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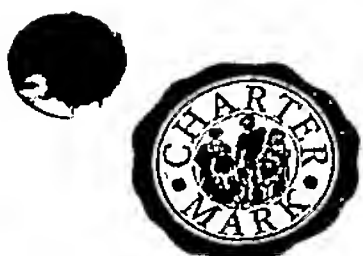
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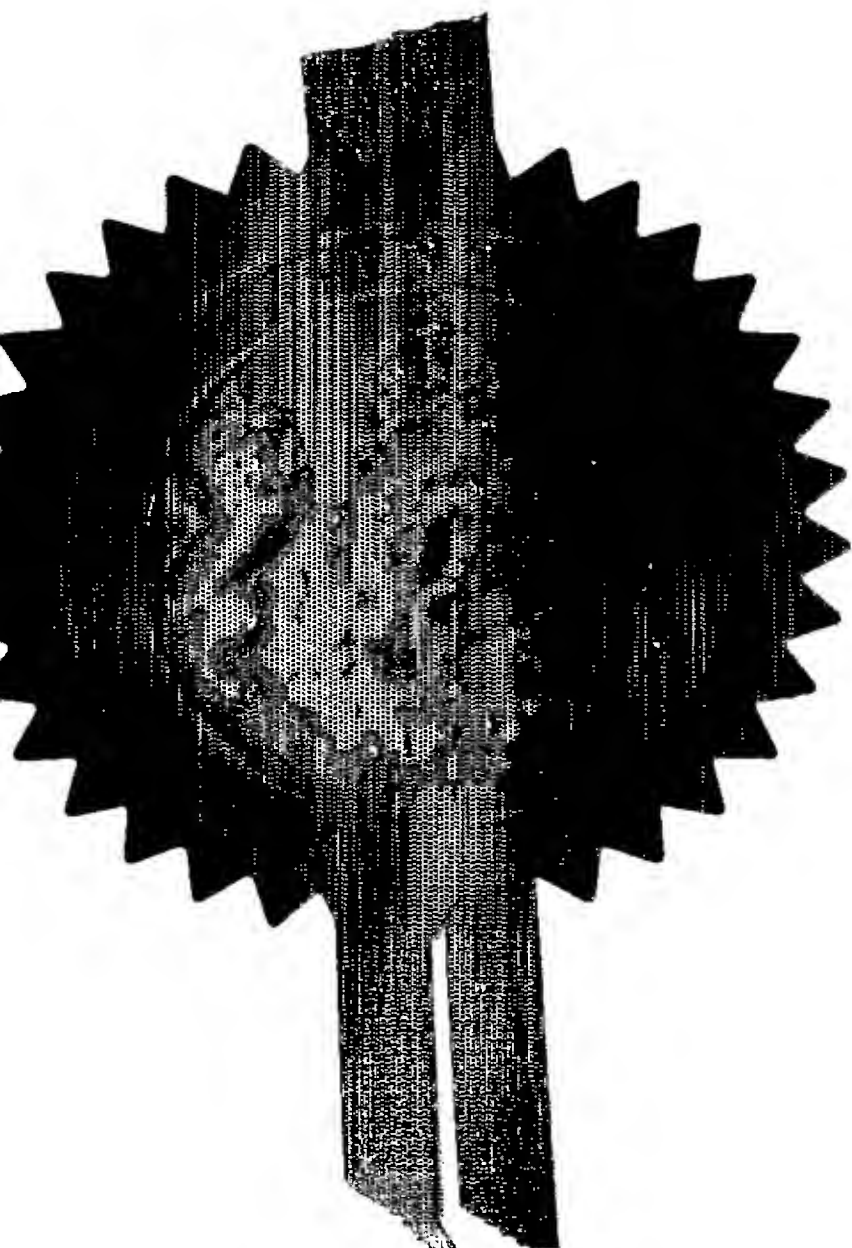
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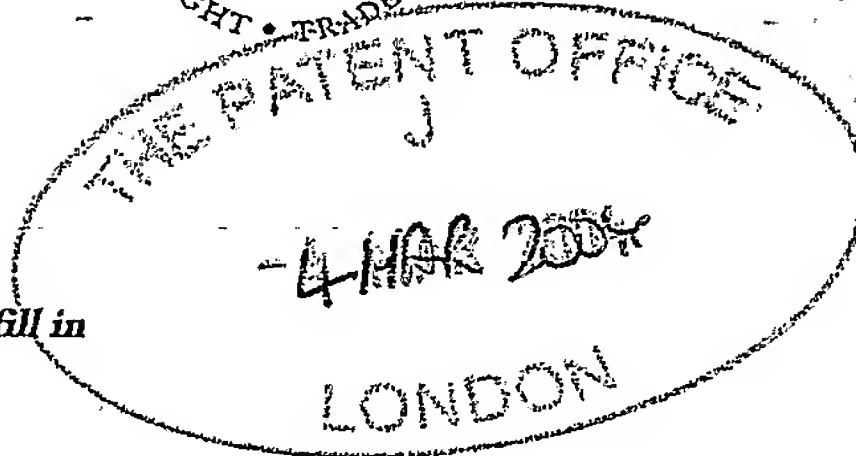




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ISIS INNOVATION LIMITED
Ewert House, Ewert Place
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Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

3998564003

4. Title of the invention

ELECTROCHEMICAL SENSORS

5. Name of your agent (*if you have one*)

J. A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)

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Priority documents

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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for a preliminary examination and search (Patents Form 9/77) 1

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J.A. KEMP & CO.

Date 4 March 2004

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

BENSON, John Everett
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ELECTROCHEMICAL SENSORS

Field of the Invention

The invention relates to electrochemical sensors for use in a non-downhole environment. In particular, it relates to sensors for the determination of pH of substances in "dirty" environments, such as effluent and other waste streams.

Background of the Invention

US Patent No. 5,223,117 relates to self-assembly microelectrodes used in electrochemical sensors. The microelectrodes (which are the working electrode in the sensors) are modified with monolayer coverages of reference and indicator molecules, with both chemically sensitive redox materials and chemically insensitive redox materials being present on the same electrode. It is necessary for the microelectrodes to be significantly smaller than the counterelectrodes, for example the counter electrode area must be at least 10^2 to 10^3 times the working electrode area. An advantage of such a small working electrode with an internal reference is that the sensor is minimally invasive, and can therefore be used in biomedical sensing.

UK Patent Application No. 2 391 314 describes electrochemical sensors for measuring the amount of hydrogen sulphide or thiols in a fluid. The sensor comprises a precursor and reaction solution which, together with the hydrogen sulphide or thiols, create a redox reaction. The current produced by this redox reaction is dependent upon the concentration of hydrogen sulphide or thiols. The sensors described in this document are for use in downhole applications, i.e. to extend down boreholes during a drilling operation. Given the size restrictions on apparatus which must extend into a borehole, the sensors must be relatively small.

However, there remains a need for more robust, reagentless sensors that can provide accurate results in hostile environments or "dirty" media such as effluents or sewage. Furthermore, there is a requirement for sensors to be used under various conditions, such as at temperatures above room temperature. There is renewed interest in developing sensors capable of measuring pH accurately at elevated temperatures. The present invention aims to address these issues.

Summary of the Invention

According to a first aspect of the invention there is provided an electrode for use in a electrochemical sensor, said electrode comprising carbon modified with a chemically sensitive redox active compound which is not anthraquinone, phenanthrenequinone or N,N'-diphenyl-*p*-phenylenediamine (DPPD).

According to a second aspect of the invention, there is provided a pH sensor comprising:

a working electrode comprising a chemically sensitive redox material; and
a counter electrode,

wherein the ratio of the surface area of the working electrode to the surface area of the counter electrode is from 1:10 to 10:1.

The invention also provide a pH sensor comprising:

a working electrode comprising a chemically sensitive redox material, and
a counter electrode,

wherein the area of the working electrode is from $500\mu\text{m}^2$ to 0.1m^2 .

These pH sensors differ from those in US 5,223,117 in the sizes of the working and counter electrodes. They have an advantage over those in the prior art in that they are less likely to be fouled or clogged by dirt in the fluid being measured, and accordingly they have a longer lifetime before they need to be replaced.

The electrodes of the present invention may be used in an electrochemical sensor, and in particular in a pH sensor. The electrodes of the invention and the pH sensors of the invention are preferably suitable for use in a non-downhole environment.

The invention also provides a method for preparing an electrode for use in an electrochemical sensor, said method comprising modifying carbon with a chemically sensitive redox active material with the proviso that the chemically sensitive redox active material is not anthraquinone, phenanthrenequinone or N,N'-diphenyl-*p*-phenylenediamine (DPPD).

Finally, the invention further provides a method for preparing an electrode *in situ* comprising applying carbon modified with a chemically sensitive redox active material to the surface of a substrate, wherein the chemically sensitive redox active material undergoes an irreversible chemical reaction when subjected to cyclic voltammetry.

Brief Description of the Figures

Figure 1 is the voltammetric response of 6-nitro chrysene carbon immobilised on bppg after the development of the reversible system at pH 1.0 (0.1 M HCl + 0.1 M KCl) varying the scan rate (25, 50, 100, 200, 300, 400, 500, 750, 1000 mV s^{-1}) and the corresponding plot of oxidative peak potential vs. scan rate (insert).

Figure 2 shows the cyclic voltammograms of PAQ at each pH studied (pH 1, pH 4.6, pH 6.8, pH 9.2 and pH 12). Step potential 2 mV, scan rate 100 mV s^{-1} .

Figure 3 is the base-line corrected oxidative and reductive square wave voltammograms of DPA at each pH studied (pH 1, pH 4.6, pH 6.8, pH 9.2 and pH 12). B) A corresponding plot of oxidative peak potential vs. pH.

Figure 4 shows cyclic voltammograms of diphenylamine derivatised carbon in pH 6.8 buffer showing the first, second and tenth scans.

Figure 5 shows cyclic voltammograms of thionin derivatised carbon particles immobilised on a bppg electrode in pH 12.0 (0.1 M NaOH + 0.1 M KCl) buffer showing the first and fourth scans.

Figure 6 shows cyclic voltammograms of 6-nitrochrysene derivatised carbon powder immobilised on a bppg electrode in pH 9.2 buffer (0.05M sodium tetraborate + 0.1 M KCl) showing the first and tenth scans. Inset: 10 CV scans 9-nitroanthracene derivatised carbon powder immobilised on a bppg electrode over the reversible systems (see text) at pH 6.8.

Figure 7 shows cyclic voltammograms of FBK derivatised carbon powder immobilised on a bppg electrode in pH 4.6 buffer (0.1 M acetic acid + 0.1 M sodium acetate + 0.1 M KCl) showing the first and second scans.

Figure 8 cyclic voltammograms of FBK derivatised carbon powder immobilised on a bppg electrode in pH 4.6 buffer (0.1 M acetic acid + 0.1 M sodium acetate + 0.1 M KCl) where the potential is cycled around system (II) only (see text) showing the first and tenth scans. Inset: CV scans of the same system with varying scan rate (25, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800 and 900 mV s^{-1})

Figure 9 shows thirty repeated CVs of azobenzene derivatised carbon at pH 4.6 showing the effect of sweeping down to very negative potentials.

Figure 10 shows baseline corrected oxidative SWV voltammograms of DPA-carbon at pH 4.6

(0.1 M acetic acid + 0.1 M sodium acetate + 0.1 M KCl) buffer over a temperature range of 25-80 °C in 5 °C increments.

Figure 11 shows a pH sensor according to the invention.

Figure 12 shows an array of pH sensors according to the invention on a single substrate.

5

Detailed Description of the Invention

The pH sensors and electrodes of the present invention are preferably used in a non-downhole environment. The term “non-downhole” means that the sensors are used in environments other than those down boreholes or other subterranean volumes of liquid. Suitable non-downhole applications include pH measurement in environmental, chemical, waste water, industrial and effluent applications. The term “environmental” includes testing such as in rivers and seas. The term “chemical” includes testing during chemical processes in laboratories or factories. The term “industrial” includes waste from industrial processes. The term “effluent” includes discharges of liquid waste, for example sewage. Effluent may be mostly or entirely non-aqueous. Waste water includes liquid waste from domestic and commercial properties. Waste water is predominantly aqueous.

In the following description the term “chemically irreversible behaviour” means that the compounds concerned react via an irreversible chemical reaction to form another species. The compound and the species into which it converts will have different electrochemical profiles. The term “chemically reversible behaviour” means that the compounds undergo reversible reactions when used in the sensors of the invention, and accordingly do not convert into other species which cannot convert back into the original compound.

The term “reversible electrochemistry” is used interchangeably with the phrase “electrochemically reversible behaviour”. This means that the compound can gain and lose electrons repeatedly without its electrochemical profile varying over time.

The individual components and aspects of the invention will now be described in more detail.

Carbon

The carbon used in the present invention must be capable of being modified by the chemically sensitive redox active material. Preferably it is in the form of carbon powder. It is particularly preferred that the carbon is in the form of graphite particles having a mean diameter of from 0.1 to 50 μm , preferably from 1 to 30 μm . Alternatively the carbon can be present in the form of carbon nanotubes. These are in effect "rolled up" sheets of graphite. Conventionally they are either single-walled carbon nanotubes or multi-walled carbon nanotubes (MWCNTs).

Chemically sensitive redox active material

10 The chemically sensitive redox active material may be any organic material capable of undergoing electron loss and gain. Preferably it is a solid phase material. When immobilised onto a substrate, e.g. glassy carbon or a basal plane pyrolytic graphite (bppg) electrode, it undergoes concomitant proton and electron loss/gain on oxidation/reduction.

15 This material is described as being "chemically sensitive" because it must show an electrochemical response which is dependent upon the species which is to be detected or measured. For example, in order to be of use in a pH sensor, the chemically sensitive redox active material must have an electrochemical response which is sensitive to a change in hydrogen ion concentration.

20 This material need not comprise only one compound, but can instead comprise a mixture of different chemically sensitive redox active compounds.

Preferred chemically sensitive redox active materials are those which comprise a) compounds exhibiting chemically and electrochemically reversible behaviour, and b) compounds exhibiting chemically irreversible behaviour leading to electrochemically reversible behaviour. Of the latter category, preferred materials comprise b.1) compounds which form polymers, and b.2) 25 compounds which contain a nitro group. Considering each of these groups in turn:

a) redox active materials comprising compounds exhibiting chemically and electrochemically reversible behaviour

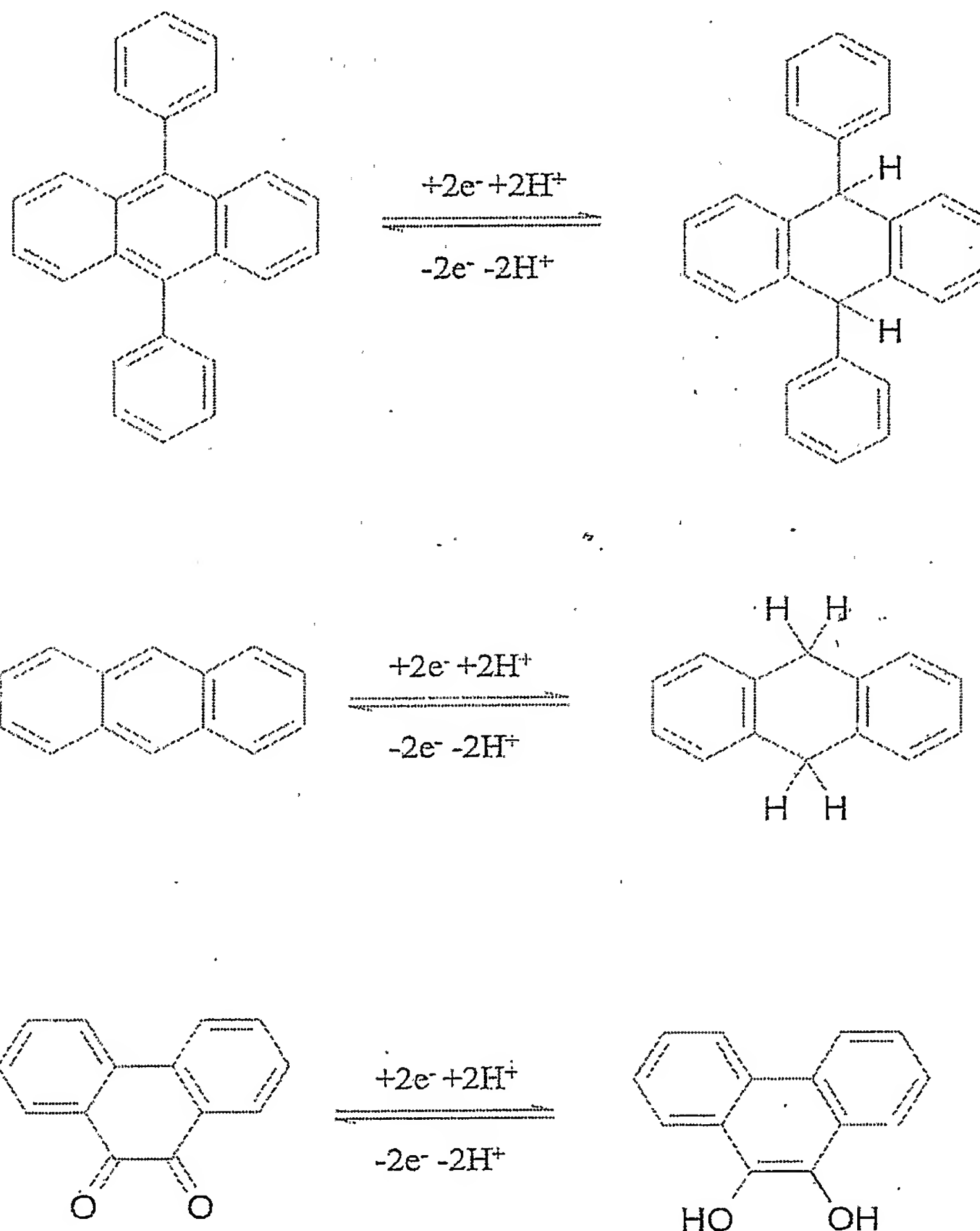
5 The compounds in these redox active materials produce stable reversible voltammetric peaks when they are subjected to both cyclic voltammetry and square-wave voltammetry. They exhibit Nernstian behaviour. When the sensor in which they are incorporated is intended to measure pH, the plot of peak potential against pH for each compound produces a linear, Nernstian response according to the following equation:

$$E_{peak} = E_{formal}^0 - \frac{2.3RTm}{nF} pH$$

10 wherein E_{peak} is the peak potential / V, E_{formal}^0 / V is the formal potential, R is the universal gas constant / J K⁻¹ mol⁻¹, T is the temperature / K, F is the Faraday constant / C mol⁻¹, and n and m are the number of electrons and protons transferred respectively. In the examples which follow, n and m are both likely to be equal to two as proposed in scheme 1. By monitoring the peak potential of these compounds, the pH can be determined.

SCHEME 1

The proposed redox pathway for DPA, PAQ and anthracene



Suitable compounds which exhibit chemically and electrochemically reversible behaviour include quinones and anthracenes. Compounds which will be effective in pH sensors are capable of undergoing a redox reaction that is reversible and involves the uptake and release of protons. The skilled person will be able to determine other compounds which will be suitable in this embodiment of the invention.

- 10 b) redox active materials comprising compounds exhibiting chemically irreversible behaviour leading to electrochemically reversible behaviour

The compounds in these redox active materials, when subjected to cyclic voltammetry, undergo

an irreversible chemical reaction. The substance formed after as a result of this irreversible chemical reaction (e.g. a different compound or a polymer of the reactant compound) exhibits electrochemically reversible behaviour similar to the compounds described in paragraph a) above. The peak potential of these substances shows a Nernstian response to the species to be measured (the target analyte) and these substances can therefore be used in electrochemical sensors such as pH sensors.

The compounds in this group can be split into a number of subsets, including:

b.1) those which form polymers

10 The compounds useful in this aspect of the invention all form polymers via an irreversible chemical reaction. The resulting polymers have peak potentials which exhibit a linear, Nernstian response to the species to be measured when subjected to cyclic voltammetry. Suitable compounds for use in this aspect of the invention include diphenylamine and phenothiazine dyes, with diphenylamine being most preferred. Suitable phenothiazine dyes include toluidine blue, 15 methylene blue and thionin. Of these, methylene blue and thionin are preferred.

In the prior art, the majority of existing amperometric pH sensors are based on the pH-switchable permselectivity of thin films and membranes. One such family of conducting polymeric films is based on polyaniline-like structures formed by electro-oxidative methods. The polymerisation of diphenylamine has been carried out electrochemically in non-aqueous solvents rather than in 20 solution via chemical means due to the poor solubility of polydiphenylamine in most solvents. Much of the discussion in the prior art has focused on the coupling mechanism for the formation of these polymers from diphenylamine in non-aqueous solvents on the surface of gold or platinum electrodes.

However, the present invention differs from the prior art in that the compounds can be used to 25 modify carbon. For example, they can be physisorbed onto the surface of carbon particles. When this occurs, the compounds undergo an oxidative electropolymerisation reaction whilst in contact with aqueous solutions to form polymers, which are themselves sensitive to changes in local pH. To apply this to a particular compound, when diphenylamine is used to modify carbon, the compound can then undergo oxidative electropolymerisation whilst in contact with aqueous

solutions to form polydiphenylamine, which is itself useful in sensing changes in local pH.

In general, the behaviour of compounds according to this aspect of the invention is as follows.

When subjected to cyclic voltammetry, an initial peak is observed, corresponding to the redox active material in its original form. Gradually this peak disappears as eventually all of the redox active material on the surface of the carbon is polymerised. A new reversible system is then generated, corresponding to the polymeric form of the redox active material. The peak corresponding to this system grows upon repetitive cycling and eventually stabilises.

By monitoring the peak potentials of both the irreversible peak and the reversible peak, the pH can be monitored. Analysis of the gradient of a plot of peak potential vs. pH for both the irreversible system and the reversible system shows a shift in a linear Nernstian fashion.

Thus, these redox active materials not only provide a robust reagentless pH sensor over a wide range, but also provide a technique for preparing an electrode *in situ*.

b.2) those which contain a nitro group

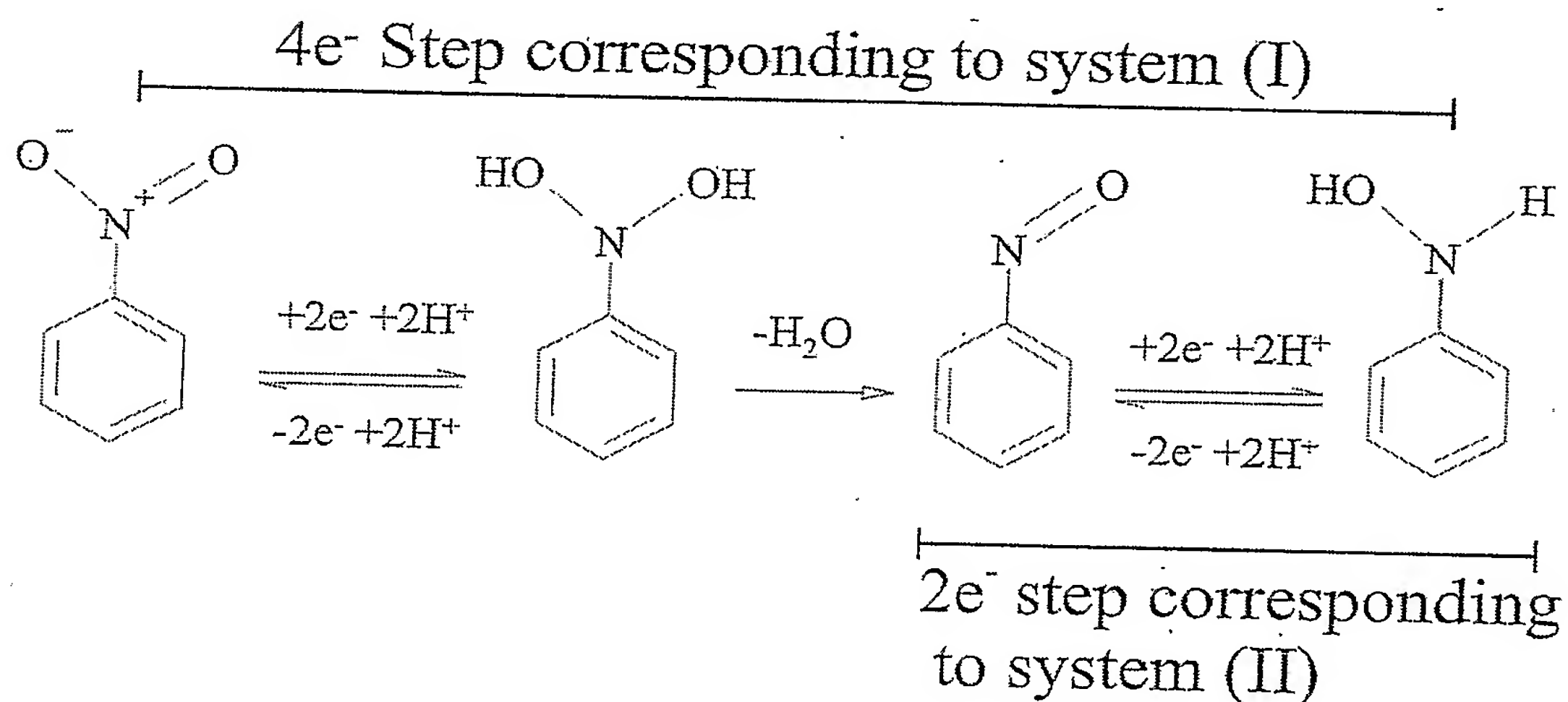
Such redox active materials are usually aromatic compounds containing one or more carbocyclic rings and/or heterocyclic rings and are substituted by at least one nitro group. Suitable carbocyclic compounds include phenyl, anthracene, fluoranthene and the like. Suitable heterocyclic compounds include the carbocyclic compounds having one or more carbon atoms in the ring replaced by one or more heteroatoms. The heteroatoms are preferably selected from nitrogen, oxygen and sulphur. Preferably the heterocyclic compounds contain, in the ring, from one to five heteroatoms, preferably from one to three heteroatoms. The compounds should preferably not undergo any other competing chemical transformations or reactions which mask their response to the species which is to be measured by the electrochemical sensors of the invention.

Preferred compounds include nitroanthracene, in particular where the anthracene molecule is substituted by the nitro group in the 9 position; nitrochrysene, in particular where the nitro group is in the 6 position; and nitrofluoranthene, in particular where the nitro group is in the 3 position. Other compounds which can be used include fast black K (FBK).

The electrochemical reductions of aromatic compounds follow complex mechanistic pathways in both aqueous and non-aqueous media, and at the three-phase boundary between microdroplets of oils containing nitro groups, aqueous electrolyte and an electrode. There has been speculation about the exact mechanism of the reduction of nitro groups, with various different pathways being suggested depending on the pH of the solution. However, it is now generally agreed that the pathway shown below in Scheme 2 below is that followed. This scheme uses nitrobenzene as an example of such a compound, but the general scheme is the same for the other nitro-containing compounds.

SCHEME 2

The general mechanism for the electrochemical reduction of an aryl nitro moiety illustrated here by nitrobenzene



In this mechanism the nitro group undergoes a six-electron, six-proton reduction to form the corresponding aryl amine which is split into two steps: a four-electron, four-proton reduction to the aryl hydroxylamine via the nitroso intermediate, and a further two-electron, two-proton reduction to the aryl amine.

As with the compounds which form polymers, these redox active materials not only provide a robust reagentless pH sensor over a wide range, but also provide a technique for preparing an electrode *in situ*.

Chemically insensitive redox active materials

In addition to the chemically sensitive redox active material, the invention may also employ chemically insensitive redox active materials. Thus, a chemically insensitive redox active material may be present on the same substrate as the chemically sensitive redox active material.

- 5 A chemically insensitive redox active material may also be present on a separate electrode used in an electrochemical sensor according to the invention.

The chemically insensitive materials may be used to modify carbon, as with the chemically sensitive materials. Alternatively, these materials can be applied directly to the substrate (to bpgg, for example). If the material is to be screen printed, then a binder and thinner would also
10 be required.

The chemically insensitive redox active materials are, like the chemically sensitive redox active materials, capable of undergoing repeated electron loss and gain. However, the electrochemical response of these compounds is not dependent on the concentration of the species to be measured by the sensor. For example, if the sensor is a pH sensor, then the electrochemical response of the
15 chemically insensitive redox active material will be insensitive to a change in concentration of hydrogen ions, and will therefore be insensitive to a change in pH.

The choice of the chemically insensitive redox compounds will clearly also depend upon the species which the electrochemical sensor is to detect. In the case of pH sensors, suitable compounds include insoluble ferricyanide or ferrocyanide salts, octacyanomolybdate (IV) salts,
20 or polymers such as polyvinylferrocene. These compounds act as reference materials, which generate their own, reference signals when subjected to cyclic voltammetry. The difference in potential between the peaks of the chemically sensitive redox compounds and the chemically insensitive redox compounds can be used to improve the accuracy of the sensor, as discussed in US 5,223,117.

25

Substrate

The substrate onto which is applied the modified carbon may be any substrate conventionally used in the manufacture of electrodes. For example, the substrate may be a basal plane pyrolytic

graphite (bppg) electrode or glassy carbon, metal electrodes such as gold or platinum, or optically transparent electrodes such as those comprising ITO. The substrate preferably has good electrical contact with the modified carbon, and also has a surface such that good coverage with the modified carbon can be achieved.

- 5 The modified carbon may be applied by any known procedure. In particular, screen-printing is a suitable conventional technique.

Working Electrode

- 10 The size of the working electrodes of the present invention sensor is such that the surface area is from $10\mu\text{m}^2$ to 0.1m^2 , preferably from $50\mu\text{m}^2$ to 0.1m^2 , and more preferably from $500\mu\text{m}^2$ to 0.1m^2 .

Another relevant parameter when considering the electrodes of the present invention is the ratio of the surface area of the working electrode to the surface area of the counter electrode. This is preferably from 1:10 to 10:1, more preferably from 1:5 to 3:1.

- 15 In contrast to the electrodes of the present invention, those disclosed in the prior art tend to have a much smaller surface area of the working electrode and/or have a counter electrode which is substantially larger in size than the working electrode.

- The electrodes of the present invention have the advantage that they are less likely to be fouled by the substances they are being used to analyse. The working life of the sensors is therefore
20 lengthened before the electrode needs to be replaced.

Furthermore, the electrodes of the invention can be made cheaply and can be readily disposed of. While being larger than those discussed in the prior art, they are still small enough to be easily carried and can thus be used in portable electrochemical sensors.

- 25 The working electrodes of the present invention may comprise one or more areas of redox active material on the same substrate. These areas are preferably separated from each other, and may thus appear as "spots" on the surface of the substrate. At least one area will comprise carbon modified with a chemically sensitive redox active material. The other area or areas may comprise chemically sensitive redox active materials or chemically insensitive redox active materials.

According to one embodiment, the working electrode comprises two separate areas, one of which comprises carbon modified with a chemically sensitive redox active material, the other of which comprises a chemically insensitive redox active material. According to another embodiment, the working electrode comprises four separate areas, two of which comprise chemically sensitive redox active materials, the other two of which comprise chemically insensitive redox active materials. One advantage of having a number of separate areas of material is that a number of electrodes can be present on one sensor, or a number of different materials can be deposited on different areas of one working electrode. The result of this is that the accuracy and sensitivity of the sensor can be increased

pH Sensor

The structure of the sensor will depend upon its final application, and hence depends upon the substance which is to be measured and the environment in which measurement will take place. Known sensor structures may be employed in conjunction with the agglomerates and electrodes described herein.

Exemplary sensors may have a two or three terminal arrangement. Thus, they may comprise a working electrode of the invention and a combined counter and reference electrode, or a working electrode, counter electrode and a reference electrode. The reference electrode and counter electrode can be any conventional electrodes known in the art, such as silver electrodes, calomel electrodes or standard hydrogen electrodes. The reference electrode can also be provided by the chemically insensitive redox active material described earlier.

The materials used in the sensor depend upon which species the sensor is intended to measure and the environment in which the sensor is to be used. In order to modify the sensor to be sensitive to a different species it is simply required for the skilled person to substitute the redox active material with a different redox active material sensitive to the species which is to be measured.

An exemplary pH sensor according to the invention is shown in Figure 11. The substrate 1 bears the electrodes 2, 3 and 4. The counter electrode 2 and the reference electrode 3 both comprise silver. In this Figure the working electrode comprises carbon derivatised with N,N'-diphenyl-*p*-

phenylenediamine (DPPD), with the electrode containing 10% by mass of DPPD. The pH sensor is connected to equipment to measure cyclic voltammetry via the three terminals 5.

Figure 12 shows another embodiment of the invention in which four different sensors are present on one base. The sensors are produced by screen printing, and can be cut into individual sensor strips when required. The numerals used in this Figure correspond to those used in Figure 11 above.

Modification Method

The preferred methods for modifying the carbon used in the present invention are

- 10 a) homogeneous chemical derivatisation with the chemically sensitive redox active material;
- b) derivatisation via physical adsorption of the chemically sensitive redox active material; and
- 15 c) physical mixing with the chemically sensitive active material and a binder.

Homogeneous chemical derivatisation describes a method wherein the carbon and chemically sensitive redox active material are combined in a solvent the presence of a reductant (e.g. hypophosphorous acid) in order to cause chemical bonding of the two species. The term "homogeneous" refers to the fact that all of the reductant (and the reductant only) is in the solution phase. The carbon is dispersed in the solvent while the reductant and the chemically sensitive redox active material (or a precursor thereof) are dissolved in solution. The chemical reaction occurs solely in the solution phase to generate a species which then bonds to the surface of the carbon.

Derivatisation via physical adsorption means that the carbon and chemically sensitive redox active material are combined in such a way as to cause the latter to become physically adsorbed onto the surface of the carbon.

The two processes above differ in that, in the case of physical adsorption, the skilled person relies on relative hydrophobicities to induce derivatisation of the carbon surface. In chemical derivatisation an actual chemical bond is formed between the carbon and the chemically sensitive

redox active material.

The physical mixing option simply requires the carbon and chemically sensitive redox active material to be mechanically mixed together in the presence of a binder. This allows the carbon and redox active material to be associated with one another without forming chemical bonds.

- 5 The choice of binder depends on the conditions to which the electrode will be subjected. Conventional binders and thinners, such as those employed in the screen-printing industry, are possible candidates.

Examples

10 Reagents and equipment

All reagents were obtained from Aldrich (except for methylene blue and thionin which were obtained from British Drug House Chemicals and potassium chloride which was supplied by Riedel de Haën) and were of the highest grade available and used without further purification. All aqueous solutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ
 15 grade water system with a resistivity of not less than 18 MΩ cm. All measurements were made after degassing the solution with pure N₂ gas (BOC gases, Guildford, Surrey, UK) for 30 minutes and unless stated otherwise results were recorded at a temperature of 22 ± 2 °C.

Solutions of known pH in the range pH 1 to 12 were made up in de-ionised water as follows:
 pH 1, 0.1M HCl; pH 4.6, 0.1 M acetic acid + 0.1 M sodium acetate; pH 6.8, 0.025M Na₂HPO₄ +
 20 0.025M KH₂PO₄; pH 9.2, 0.05M disodium tetraborate; pH 12, 0.01M sodium hydroxide. These solutions contained in addition 0.1M KCl as additional supporting electrolyte.

pH measurements were performed on each freshly made solution to ensure it had the correct pH using a Jenway 3030 pH meter.

Electrochemical measurements were recorded using a μAutolab computer controlled potentiostat
 25 (Ecochemie, Netherlands) with a standard three-electrode configuration. All room-temperature experiments were carried out in a cell of volume 30 cm³. High-temperature voltammetry (30-70 °C) was undertaken using a double-walled glass cell of volume 25 cm³ thermostatted to the desired temperature through circulation of water from a heated water bath. In all cases a basal plane pyrolytic graphite (bpgg, 0.20 cm², Le Carbone Ltd., Sussex, UK) electrode acted as

the working electrode (see below). A platinum rod acted as the counter electrode, and a saturated calomel electrode as the reference electrode (SCE, Radiometer, Copenhagen) completed the cell assembly.

Unless stated otherwise cyclic voltammograms were recorded using the following parameters:
 5 step potential 2 mV, scan rate 100 mV s⁻¹. Square wave voltammetric parameters were as follows: frequency 12.5 Hz, step potential 2 mV and amplitude 5 mV.

Scanning electron microscopy (SEM) was conducted using a Cambridge stereoscan electron microscope at a magnification of 83x. Initial characterisation of the size of the carbon particles was carried out using scanning electron microscopy (SEM). This involved attaching the carbon
 10 particles to a strip of conducting sticky tape, from which the SEM image was taken. Analysis of the image revealed that the carbon particles had a mean diameter of 1.5 µm, consistent with that stated by the manufacturer (Aldrich, graphite powder, 1-2 µm, synthetic).

Example 1: Carbon powder derivatisation methods

15 a) Derivatisation via Physical adsorption:

Physical adsorption onto carbon powder was carried out by mixing 2 g of carbon powder with 25 cm³ 0.1M HCl + 0.1M KCl and 10 cm³ of a 10mM solution in acetone of one of the following compounds: anthracene, azobenzene (AB), diphenylamine, 9,10-diphenylanthracene (DPA), 1,3-diphenyl guanidine, fluorescein, methylene blue, 3-nitrofluoranthene (3-NF), 6-nitrochrysene
 20 (6-NC), 9-nitroanthracene (9-NA), 9,10-phenanthraquinone (PAQ) or triphenylamine. The reaction mixture was stirred continuously for 2 hours in a beaker and then filtered by water suction after which it was washed with distilled water to remove the acid and salt. It was then air-dried by placing inside a fume hood for 12 hours and finally stored in an airtight container.

25 b) Homogeneous chemical derivatisation

Initially 2 g of carbon powder was mixed with a 10 cm³ solution containing 5mM Fast Black K (2,5-dimethoxy-4-[(4-nitrophenyl)azo]benzenediazonium chloride; FBK), to which 50 cm³ hypophosphorous acid (H₃PO₂, 50%; Aldrich) was added. The reaction mixture was then left to stand at 5 °C for 30 minutes with stirring every ten minutes, after which the solution was filtered

by water suction in order to remove any unreacted species from the carbon. Further washing with deionised water was carried out to remove any remaining acid and finally with acetonitrile to remove any unreacted diazonium salt from the mixture. The carbon particles were then air-dried by placing inside a fume hood for a period of 12 hours after which they were stored in an airtight container.

Lifetimes of the derivatised carbon powders:

Each compound derivatised using one of the methods given above and stored in an airtight container was studied over a period of several months and was found to produce stable voltammograms after this period of time had elapsed. This shows that there is little or no desorption from the carbon particle surface and that the derivatised carbon powders are stable over time

Example 2: Immobilisation of the derivatised carbon onto a substrate

The newly derivatised carbon powders were characterised by abrasive immobilisation onto the surface of a bppg electrode prior to characterisation. This was done by initially polishing the electrode on glass polishing paper (H00/240) after which they were polished on silicon carbide paper (P1000C) for smoothness. The derivatised carbon was then mechanically immobilised onto the bppg electrode by gently rubbing the electrode surface on a fine filter paper (Whatman) containing the functionalised carbon. It is worth noting that in the case of 3-nitrofluoranthene, 6-nitrochrysene and 9-nitroanthracene derivatised carbon powders, the derivatised carbon was immobilised onto the basal plane at the beginning of each set of experiments as the electro-reduction of the nitro group is chemically irreversible and hence the signal is lost after the first initial scan (see below).

Characterisation protocol:

In order to verify that each compound studied was attached to the carbon particles, either through physical adsorption or via a covalent bond depending on the derivatisation method, the following protocol was carried out using cyclic voltammetry over the entire pH range studied (pH1-12) on

each newly derivatised carbon immobilised onto a bppg electrode. First ten repetitive scans (not shown) from +1.0 V to -1.0 V were typically conducted to ensure the stability of the species. In each case an electrochemically reversible system could be observed which rapidly stabilised to give a nearly symmetrical wave shape with a separation of ca 20 mV between the oxidative and reductive peaks which is close to the ideal zero peak to peak separation for an immobilised species. Next the electrolyte solution was replaced with fresh solution and the voltammetric response recorded. The corresponding voltammetric response (not shown) was found to overlay the last scan thereby confirming that the electroactive species remains on the electrode surface. Finally the scan rate was varied and a plot of peak current vs. scan rate was found to be linear, consistent with a surface bound species.

Together these tests each confirm that a particular compound studied is attached to the surface of the carbon particles. By way of an example Figure 1 shows the voltammetric response of 6-nitrochrysene physically derivatised carbon with varying scan rate (25-1000 mV s⁻¹) at pH 1.0 (0.1M HCl + 0.1M KCl) after development of the reversible system (see below). Inset is a plot of the corresponding peak current against scan rate which is linear as expected for a surface bound species.

All of the compounds studied were found to be immobilised and stable between pH 1-12 at room temperature except for thionin and methylene blue which were both found to slowly desorb upon repetitive cycling at pHs greater than 6.8 and 4.6 respectively. Fluorescein, 1,3-diphenyl guanidine and triphenylamine produced very poorly defined voltammetric waves at any pH and as such no further analysis was performed on them.

Example 3: Voltammetric response of the derivatised carbons at 22 °C from pH 1 to 12

The response of the derivatised carbons at each pH was first studied individually using cyclic voltammetry (CV) and then using square wave voltammetry (SWV). SWV was utilised as the electrochemical probe of the system as it has significant advantages to conventional CV, providing well-defined voltammetric peaks in a single sweep due to the reversibility of each redox system studied. The corresponding cyclic voltammograms and square wave voltammograms were recorded in a range of pH solutions (pH 1.0, 0.1M HCl + 0.1M KCl; pH 4.6, 0.1M acetic acid + 0.1M sodium acetate + 0.1M KCl; pH 6.8, 0.025M Na₂HPO₄ + 0.025M

KH_2PO_4 + 0.1M KCl; pH 9.2, 0.05M disodium tetraborate + 0.1M KCl; pH 12, 0.01M KOH + 0.1M KCl). The voltammetric behaviour of compounds of the invention can be grouped into three types: (1) chemically and electrochemically reversible behaviour, (2) chemically irreversible leading to electrochemically reversible systems involving the formation of polymeric species and (3) chemically irreversible leading to electrochemically reversible systems involving nitro containing compounds.

Example 3.1: Compounds displaying chemically and electrochemically reversible behaviour

The voltammetric response of graphite powder derivatised with PAQ and DPA according to the method in Example 1 was measured. Figure 2 shows the overlaid cyclic voltammetric responses of PAQ measured over the pH range 1-12. The peak shapes are nearly symmetrical with a slight separation of ca 20 mV between oxidative and reductive peaks at each pH. A slight shoulder at higher potential can be observed on each peak which was not observed with either DPA or anthracene. This is analogous to the voltammetry observed when anthraquinone is derivatised onto carbon powder and can be tentatively attributed to intermediate reduction/oxidation of the quinone/semi-quinone species respectively.

Figure 3A shows the overlaid oxidative and reductive SWV response of DPA over the pH range 1-12 and figure 3B shows the corresponding plot of peak potential against pH. This plot clearly shows a linear response to pH with a gradient of 61 mV/pH unit which is in excellent agreement with theory (equation 1). A comparison of the experimentally obtained potential shifts with pH for each compound with theory is given in Table 1 for each compound in this class.

Table 1

Comparison between theoretically calculated shift in peak potential with pH (58.1 mV/pH unit, equation 1) and experimentally determined shifts of peak potential with pH for anthracene, DPA and PAQ taken from oxidative SWV scans at 22 ± 2 °C.

Compound	Experimental Shift ± 2 (mV/pH unit)
Anthracene	57.5
9, 10-Diphenylanthracene	61.6
9, 10-Phenanthraquinone	56.3

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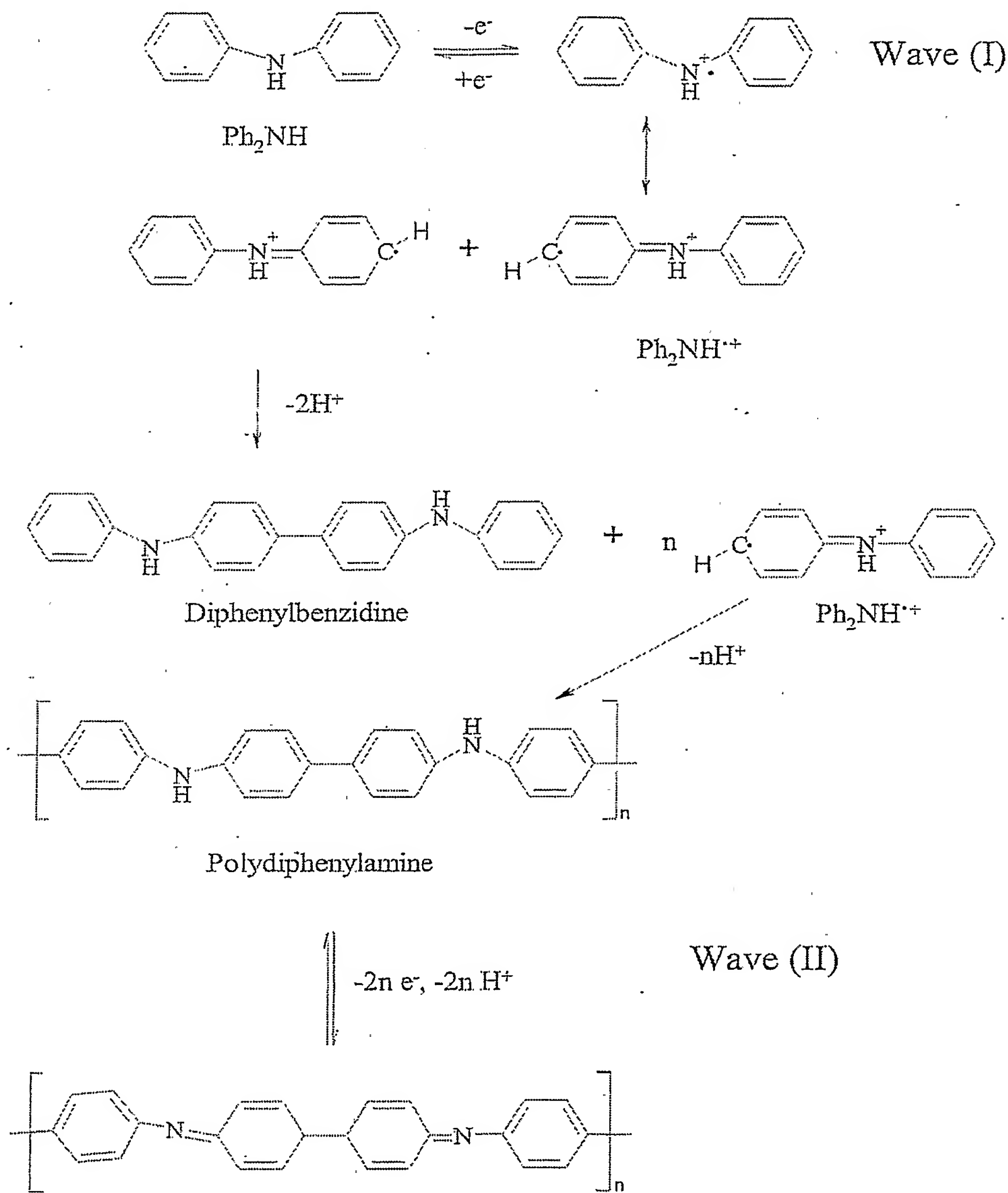
Example 3.2: Compounds displaying chemically irreversible behaviour leading to electrochemically reversible behaviour – compounds which form polymers

Cyclic voltammetry of carbon powder derivatised with diphenylamine revealed that upon first scanning in an oxidative direction a large electrochemically irreversible wave is observed at ca +0.45 V vs. SCE at pH 6.8 (figure 4). Upon reversing the scan direction at +1.0 V a new wave is observed at ca +0.03 V vs. SCE which upon repetitive cycling grew to give a stable, reversible redox system, whilst the large peak at +0.45 V died away after 4 cycles. This behaviour is analogous to that reported in the literature for electropolymerisation of diphenylamine in solution except that in this case the polymerisation occurs for diphenylamine physisorbed onto the surface of carbon particles in contact with an aqueous solution. The large electrochemically irreversible peak labelled (I) in figure 4 can be attributed to the oxidation of diphenylamine to its corresponding radical cation and subsequent polymerisation via a mechanism involving concomitant proton loss and gain as shown in scheme 3.

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Scheme 3

The proposed electropolymerisation mechanism for diphenylamine



- 5 Upon repetitive cycling peak (I) disappears as eventually all the diphenylamine on the surface of the carbon is polymerised. A new reversible system, labelled as (II) in figure 4, grows upon repetitive cycling and stabilises. This can be tentatively attributed to the redox response of the polydiphenylamine involving subsequent oxidation/reduction of the imine linkages in the polymer structure and subsequent proton loss/gain (scheme 3). It is worth noting that in non-

aqueous media the polymerisation is thought to begin with the dimerisation of diphenylamine to form diphenylbenzidine and two corresponding reversible waves were reported at a higher potential than those corresponding to the polymeric species. In the present case two small reversible waves are observable at ca +0.25 V for the first few scans. These are tentatively
 5 attributed to oxidation/reduction of diphenylbenzidine, but these too die away and by the tenth scan they can hardly be observed as the dimer is further polymerised to form polydiphenylamine (scheme 3).

It was found that this voltammetric response was characteristic of diphenylamine derivatised carbon at each pH studied from pH 1-12 and that the peak potentials of both peak (I) and peaks
 10 (II) shifted in a negative direction as predicted from equation (1). Analysis of the gradient of a plot of peak potential for both the irreversible system (I) and the reversible system (II) against pH (not shown) found that each system shifted by 56 mV/pH unit and 66 mV/pH unit respectively in a linear, Nernstian fashion over the entire pH range. This suggests that carbon particles derivatised with diphenylamine which subsequently undergoes electropolymerisation to form
 15 polydiphenylamine provide not only a robust reagentless pH sensor over the pH range 1-12, but also a novel technique to prepare an electrode *in situ* in an aqueous environment.

Example 3.3: Compounds displaying chemically irreversible behaviour leading to electrochemically reversible behaviour – compounds which form polymers

20 Another class of polymeric films is derived from phenothiazine dyes such as toluidine blue, and importantly in the present context, methylene blue and thionin. These molecules are often used as mediators in amperometric sensors coated in Nafion films that detect biologically active molecules and enzymes such as nicotinamide adenine dinucleotide (NADH) and β -d-glucose. The redox properties of methylene blue and thionin derivatised carbon particles are more
 25 complicated than that of diphenylamine-carbon and very much dependent on pH. In solution the number of electrons transferred (n) for both methylene blue and thionin is reported to always be equal to two but the number of protons transferred (m) is reported to vary with pH such that $m=3$ at $\text{pH} < 5.4$, $m=2$ at $\text{pH } 5.4 < 6.0$ and $m=1$ at $\text{pH} > 6.0$.

During the characterisation of both methylene blue and thionin derivatised carbon particles using
 30 cyclic voltammetry, it was observed that below a certain oxidising potential (which depended on

pH but varied from +1.2 V at pH 1.0, +1.0 at pH 4.6, +0.65 V at pH 9.2 to +0.4 V at pH 12.0 vs. SCE) reversible waves were observed corresponding to the oxidation/reduction of the monomeric species. If the potential was swept beyond this oxidising potential a new wave was observed which has been described in the literature as corresponding to the oxidative electropolymerisation of methylene blue or thionin respectively (figure 5). Upon reversal of the scan direction the peaks corresponding to the reduction of the monomeric species were absent. On repetitive cycling a broad, low, undefined wave at a potential ca 0.2 V more positive than that corresponding to the monomer was observed which is analogous to that reported in the literature and has been attributed to the redox properties of the polymer (figure 5). Below pH 4.6 the variation of peak potential of the monomeric species with pH was found to be 86 mV/pH unit and 83 mV/pH unit for methylene blue and thionin respectively; above pH 6.8 the shift in peak potential with pH was found to be 33 mV/pH unit and 33 mV/pH. This is analogous to the behaviour reported in the literature for both species in solution. This demonstrates that carbon particles can successfully be modified by methylene blue and thionin, and that the resulting modified particles are useful in electrochemical sensors.

Example 3.4: Compounds displaying chemically irreversible behaviour leading to electrochemically reversible behaviour – compounds which contain nitro groups

The behaviour of 9-NA, 6-NC and 3-NF derivatised carbon powder can be generically characterised by discussing the voltammetry observed for 6-NC. Where differences in behaviour between compounds arise they will be discussed.

Upon first scanning the freshly immobilised 6-NC in a reductive direction from +1.0 V to -1.0 V vs. SCE at pH 9.2 a large, reductive peak was observed at -0.75 V (labelled as (I) in figure 6). Upon reversing the scan direction at -1.0 V no reverse peak was seen but a new oxidative peak (labelled as (II) in figure 6) was observed at ca -0.25 V and a low, broad oxidative wave was also observed at ca 0.25 V. Upon repetitive cycling the electrochemically reversible system at -0.25 V stabilised while the electrochemically irreversible reductive wave at -0.75 V rapidly died away (figure 6). In the case of 9-NA a further reversible wave at ca -0.55 V also grew with repetitive cycles labelled as (III) in figure 6 (insert). The reductive wave in the system at -0.25 V has a pronounced shoulder on it, this will be discussed below. Both waves I and II (and III in the case

of 9-NA) were found to be present at each pH in the range 1 to 12 and shifted in a Nernstian, linear fashion (where n is equal to m in equation (1) and is likely to be equal to four for the system labelled (I) and two for system (II)) for all three compounds 9-NA, 6-NC and 3-NF. Table 2 details the peak potentials of each system I and II for each compound studied at pH 6.8 for comparison while Table 3 details the shift of each peak with pH for each compound studied.

Table 2

A comparison of the peak potentials of system (I) corresponding to the six-electron, six-proton nitro group reduction and system (II) corresponding to the aryl hydroxylamine/aryl nitroso redox system for 9-NA, 6-NC and 3-NF at pH 6.8

Compound	System (I) Peak Potential / V	System (II) Oxidative Peak Potential / V	System (II) Reductive Peak Potential / V
9-Nitroanthracene	-0.722	-0.402	-0.428
6-Nitrochrysene	-0.654	-0.210	-0.254
3-Nitrofluoranthene	-0.785	-0.107	-0.150

Table 3

A comparison between theoretically calculated shift in peak potential with pH (58.1 mV/pH unit, equation 1) and experimentally determined shifts of peak potential with pH for system (I) and system (II) for 9-NA, 6-NC and 3-NF taken from oxidative SWV scans at 22 ± 2 °C.

Compound	Experimental Shift ± 2 (mV/pH unit)	
	System (I)	System (II)
9-NA	54.3	53.2
6-NC	53.5	52.2
3-NF	56.4	61.3

The origin of each wave will be discussed in turn but first it is worth reiterating the fact that for every compound studied the characterisation protocol discussed above was carried out on the system labelled (II) after several scans had been performed to stabilise the system. In every case the results of all three tests (many repeat scans giving a stable symmetric wave, replacement of the buffer solution with fresh solution and a linear relationship between peak current and scan rate) described in the protocol above confirmed that each compound was attached to the surface of the carbon particles at each pH studied from pH 1 to pH 12.

Comparison with the literature reveals that the voltammetric response of all the compounds studied which is described above and shown in figure 6 is characteristic of the electrochemical reduction of an aromatic molecule containing a nitro group and is consistent with the general mechanism shown in scheme 2.

By analogy with the reduction of nitrobenzene peak (I) corresponds to the four-electron, four-proton reduction of the nitro moiety to the corresponding arylhydroxylamine, which involves a two-electron, two-proton chemically irreversible reduction followed by a further two-electron, two-proton step.

Upon subsequent cycles the irreversible system at ca 0 V (pH 6.8) labelled as "polymerisation" in figure 6 can be tentatively ascribed to the formation of oligomers by the electro-oxidation of the aryl amine moiety (formed by sweeping the potential past system (I) and further reducing the arylhydroxylamine to the corresponding aryl amine) to its corresponding radical cation and subsequent polymerisation. This wave also rapidly dies away as all remaining aryl amine species on the surface of the carbon is polymerised to form what is apparently an electro-inactive polymer.

The reversible system labelled as (II) in figure 6 grows and stabilises after 10 scans at each pH. Again this is characteristic of the voltammetry reported in the literature and can be attributed to the chemically and electrochemically reversible two-electron, two-proton oxidation/reduction of the aryl hydroxylamine/aryl nitroso moieties. This system remains stable as long as the potential is not swept to very reducing values (ca -1.2 V at pH 6.8) whereupon the peaks gradually decrease due to some of the aryl hydroxylamine being further reduced irreversibly to the corresponding amine. A pronounced shoulder is observed at some pHs on this system, particularly in the case of 9-NA and this may possibly be due to intermediate oxidation/reduction

of the aryl hydroxylamine/aryl nitro moiety.

In the case of 9-NA, the system labelled as (III) in figure 6 (insert) at a more negative potential than the reduction of the nitroso to hydroxylamine moiety is not characteristic of a nitro reduction. However, comparison of the voltammetric behaviour of anthracene discussed above may reveal a clue as to its identity. Comparison of the peak potentials of system (III) and its shift with pH match that of the reversible system observed in the voltammetry of anthracene which undergoes a two-electron, two-proton ring reduction at the 9 and 10 positions shown above in scheme 1.

A two-electron, two-proton reduction at the 9 and 10 position in 9-NA is still possible, although the presence of the electron withdrawing nitro group may affect the redox potential slightly. Furthermore, it was also observed that as the pH was increased from pH 1 to pH 6.8 that the magnitude of the peak current also decreased corresponding to a decrease in the local proton concentration.

Example 3.5: Compounds displaying chemically irreversible behaviour leading to electrochemically reversible behaviour – compounds which contain nitro groups

The voltammetry of FBK (2, 5-dimethoxy-4-[(4-nitrophenyl)azo]benzenediazonium chloride) derivatised carbon was also investigated. In this compound the reduction of the nitro group is further complicated by the presence of an azo linkage. Initially a reductive scan was performed using cyclic voltammetry at each pH. Two irreversible peaks were observed (figure 7), the first at higher potential (labelled as (II) in figure 7) is as yet unidentified (see below) while the latter at more negative potential is characteristic of the now familiar four-electron, four-proton reduction of the nitro group (labelled as (I) in figure 7). However the voltammetry of FBK differs from the compounds discussed above because on reversing the scan direction at -1.0 V no reverse peak was observed for the nitro reduction as expected, but no new oxidative peaks corresponding to the hydroxylamine moiety were observed (although there is again some evidence of possible polymerisation due to the amine being oxidised to its radical cation as a low, broad peak is observed above 0 V with the exact potential dependant on pH.) Upon subsequent repeat cycles no peaks, either reductive or oxidative are observed at any pH. However, each system, (I) and (II), was found to shift linearly and in a Nernstian fashion with pH. The nitro system shifted by

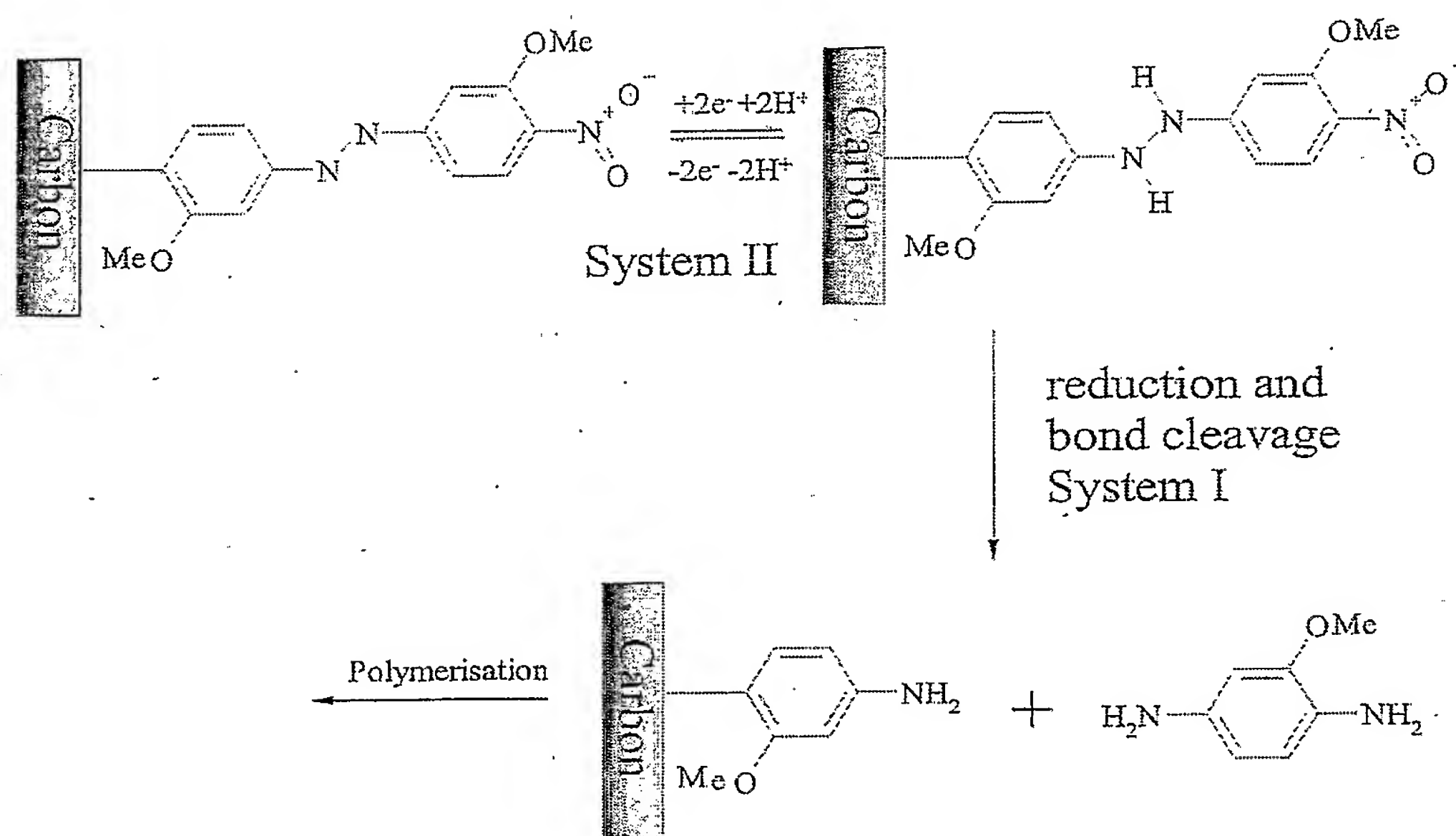
57 mV/pH unit while system (II) shifted by 61 mV/pH unit. In order to understand the electrochemistry further experiments were undertaken using CV where the potential was swept in a negative direction as far as the first unidentified system where upon the scan direction was reversed just after a peak had been observed. It was found that this produced a stable reversible system which, when the characterisation protocol was performed upon it, confirmed that the FBK was derivatised onto the carbon particles consistent with other studies of diazonium salts derivatised onto carbon through a chemical bond. Figure 8 shows this reversible system at pH 4.6 and inset shows the cyclic voltammograms with varying scan rate used in the characterisation protocol. It is only when the potential is swept beyond system (II) and the nitro reduction corresponding to (I) occurs that all subsequent signals in repeat scans are lost. Having confirmed that the FBK was immobilised onto the carbon surface and was not desorbing, another explanation for this behaviour was sought.

Azobenzene was then derivatised onto carbon particles, and it was verified that it was indeed physisorbed onto the carbon surface using the characterisation protocol described above. Cyclic voltammetry at each pH was recorded. Figure 9 shows the results of thirty cycles for azobenzene-carbon at pH 4.6. A reversible system is observed with a peak separation of 130 mV at ca -0.3 V which is close to the potential observed for system (II). Furthermore it can be seen that although the azobenzene was physisorbed onto the surface of the carbon and was not observed to desorb at any pH, if the potential is swept to very negative potentials of ca -1.0 V vs. SCE the azobenzene peak is seen to gradually die away. One possible explanation for the large peak separation is that protonation effects influence the redox kinetics of adsorbed films of azobenzene such that the reaction kinetics are sluggish and quasi-reversible when compared to azobenzene in solution where they take on more reversible character. At pH 1.0 no reverse (oxidative) peak is observed implying that the reduced form of the azo moiety is either irreversibly protonated, or the protonation induces cleavage of the azo linkage. This cleavage is also a likely explanation for the gradual loss of any voltammetric signal from the azobenzene-carbon if the potential is scanned to very negative potentials as the N-N bond may be further reduced.

Given these results, one hypothesis that explains the behaviour of FBK carbon is given in scheme 4.

Scheme 4

The proposed mechanistic pathway for the electrochemical reduction of FBK derivatised carbon powder



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Initially scanning in a negative direction from +1.0 V vs. SCE first the azo linkage is reduced in a two-electron, two-proton step to the corresponding hydrazo form which gives a corresponding Nernstian shift in peak potential with pH as is observed experimentally. If the potential is then reversed the corresponding oxidative process occurs and the system behaves reversibly and is stable over many scans. However, if the potential continues to be swept to more reducing values then the nitro group is reduced which also leads to the hydrazo-link being cleaved due to nitro reduction occurring at such negative potentials. Upon reversal of the scan direction no oxidation peaks corresponding to either the nitro group (as expected) or the azo linkage (because it has been cleaved) are observed. However a large broad wave that is characteristic of amine polymerisation is observed between 0.0 to +0.4V depending on pH. After which no further redox processes are observed in any of the repeated voltammograms. This hypothesis is supported by the mechanistic studies of Heyrovský *et al.* whose polarograms are consistent with a mechanism involving reduction of a nitro group with subsequent cleavage of a hydrazo-linkage. Furthermore the peak area of the nitro reduction peak was always found to be significantly greater than six times the area of the reduction peak corresponding to system (II), which by itself is not proof that

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this mechanism is correct but certainly provides further support in favour of hydrazo cleavage occurring after the nitro group reduction.

It has thus been demonstrated that despite the complicated mechanisms, product interference and other substituent group interactions when nitro compounds in accordance with the invention are reduced, a large and clearly resolved irreversible peak corresponding to the four-electron, four-proton reduction of the NO_2 moiety can be observed. The peak potential of this peak shifts in a linear Nernstian fashion with pH as detailed in Table 3. These compounds derivatised onto carbon powder therefore present ideal candidates from which to manufacture so-called "single-shot" disposable reagentless pH sensors for use in environments where a disposable sensor may be preferred to a reusable one, such as in sewage and other unpleasant effluents.

Example 4: pH tests at elevated temperatures

In this example, the response of four compounds to pH at temperatures ranging from 20 °C to 70 °C was studied. The experimental results obtained were varied with those predicted theoretically using equation 1.

It can be seen from equation (1) that as the temperature is increased, so too does the gradient of a plot of peak potential against pH. A further point to consider is how the pH of a solution will vary with temperature as the dissociation constants of the components of the buffer solution vary as the temperature is changed. Therefore the response of PAQ, DPA, anthracene and 9-NA to pH at elevated temperatures was studied using four IUPAC buffers (pH 1.5, 0.1M potassium tetraoxalate + 0.1M KCl; pH 4.6, 0.1M acetic acid + 0.1M sodium acetate + 0.1M KCl; pH 6.8, 0.025M Na_2HPO_4 + 0.025M KH_2PO_4 + 0.1M KCl; pH 9.2, 0.05M disodium tetra borate + 0.1M KCl) which have a known pH at each temperature studied.

Figure 10 shows the effect of temperature on the SWV voltammetry of DPA at pH 4.6 showing that elevated temperatures produce enhanced peak currents. It is also worth noting that the peak potential shifts in a negative direction with increasing temperature as predicted by equation (1). This behaviour is characteristic of all the compounds selected for investigation at high-temperature. Table 4 details the shift of each compound with pH at each temperature and compares them to the theoretical predictions of equation (1). Good agreement is found over the entire temperature and pH range between theory and experiment thus showing that carbon

powders derivatised with a variety of different compounds can be used as reagentless pH sensors from pH 1-9 at elevated temperatures up to 70 °C.

Table 4

- 5 A comparison between theoretically calculated (equation 1) and experimentally determined shifts of peak potential with pH for anthracene, DPA and PAQ taken from oxidative SWV and 9-NA (nitro reduction wave) taken from CV voltammograms over the temperature range 20-70 °C.

Temperature (°C)	Theoretical Shift (mV/pH unit)	Experimental Shift ± 2 (mV/pH unit)			
		Anthracene	DPA	PAQ	9-NA
20	58.1	57.5	61.6	56.3	52.8
30	60.1	64.8	59.2	55.3	50.0
40	62.1	66.1	60.6	57.2	57.8
50	64.1	65.3	61.4	61.2	53.7
60	66.1	65.2	62.2	61.2	52.2
70	68.1	65.9	61.2	62.0	60.2

- 10 It will be apparent to those skilled in the art that modifications may be made to the invention as described above without departing from the scope of the claims below.

Claims

1. An electrode for use in a electrochemical sensor, said electrode comprising carbon modified with a chemically sensitive redox active compound which is not anthraquinone, phenanthrenequinone or N,N'-diphenyl-*p*-phenylenediamine (DPPD).
2. An electrode according to claim 1 wherein the carbon is modified by one or more of the following methods:
 - 1) homogeneous chemical derivatisation with the chemically sensitive redox active material;
 - 2) derivatisation via physical adsorption of the chemically sensitive redox active material; and
 - 3) physical mixing with the chemically sensitive redox active material and a binder.
3. An electrode comprising carbon modified with a redox active material, wherein the redox active material undergoes an irreversible chemical reaction when the electrode is subjected to cyclic voltammetry.
4. A pH sensor comprising:
 - a working electrode comprising carbon modified with a chemically sensitive redox active material; and
 - a counter electrode,wherein the ratio of the surface area of the working electrode to the surface area of the counter electrode is from 1:10 to 10:1.
5. A pH sensor according to claim 4 wherein the surface area of the working electrode is from $10\mu\text{m}^2$ to 0.1m^2 .

6. A pH sensor according to claim 5 wherein the surface area of the working electrode is from $50\mu\text{m}^2$ to 0.1m^2 .

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7. A pH sensor comprising:

a working electrode comprising carbon modified with a chemically sensitive redox active material, and
a counter electrode,

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wherein the area of the working electrode is from $500\mu\text{m}^2$ to 0.1m^2 .

8. A pH sensor according to any one of claims 4 to 7 wherein the ratio of the surface area of the working electrode to the surface area of the counter electrode is from 1:5 to 3:1.

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9. A pH sensor according to any one of claims 4 to 8 wherein the surface area of the working electrode is from 0.5mm^2 to 10mm^2 .

10. A pH sensor according to any one of claims 4 to 9 wherein the chemically sensitive redox active material is sensitive to a change in pH.

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11. A pH sensor according to claim 10 wherein the carbon is modified by one or more of the following methods:

1) homogeneous chemical derivatisation with the chemically sensitive redox active material;

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2) derivatisation via physical adsorption of the chemically sensitive redox active material; and

- 3) physical mixing with the chemically sensitive redox active material and a binder.

12. A pH sensor according to any one of claims 4 to 11 wherein the working electrode further
5 comprises at least chemically insensitive redox active material.

13. A pH sensor according to any one of claims 4 to 12 wherein the chemically sensitive redox active material comprises more than one different compound.

10 14. A pH sensor according to claim 13 wherein the working electrode comprises two redox active materials which are sensitive to a change in pH and two redox active materials which are insensitive to a change in pH.

15 15. A pH sensor according to any one of claims 10 to 14 wherein the chemically sensitive redox active material undergoes an irreversible chemical reaction when the electrode is subjected to cyclic voltammetry.

20 16. A pH sensor according to claim 13 wherein the product of the irreversible chemical reaction displays reversible electrochemistry when the electrode is subjected to cyclic voltammetry.

17. Use of an electrode as described in any one of claims 1 to 3 in an electrochemical sensor.

18. Use according to claim 17 wherein the sensor is a pH sensor.

25 19. Use of a pH sensor according to any one of claims 4 to 16 in a non-downhole environment.

20. A method for preparing an electrode for use in an electrochemical sensor, said method comprising modifying carbon with a chemically sensitive redox active material with the proviso that the chemically sensitive redox active material is not anthraquinone, phenanthrenequinone or N,N'-diphenyl-*p*-phenylenediamine (DPPD).
21. A method according to claim 20 wherein the step of modifying the carbon comprises one or more of the following methods:
- 1) homogeneous chemical derivatisation with the chemically sensitive redox active material;
 - 2) derivatisation via physical adsorption of the chemically sensitive redox active material; and
 - 3) physical mixing with the chemically sensitive redox active material and a binder.
22. A method according to claim 20 or 21 wherein the chemically sensitive redox active material undergoes an irreversible chemical reaction when subjected to cyclic voltammetry.
23. A method according to claim 22 wherein the product of the irreversible chemical reaction displays reversible electrochemistry when the electrode is subjected to cyclic voltammetry.
24. A method according to any one of claims 20 to 23 further comprising the step of applying the carbon modified with the chemically sensitive redox active material to a substrate.
25. A method according to any one of claims 18 to 24 wherein the chemically sensitive redox active material is sensitive to a change in concentration of hydrogen ions.

26. A method for preparing an electrode *in situ* comprising applying carbon modified with a chemically sensitive redox active material to the surface of a substrate, wherein the chemically sensitive redox active material undergoes an irreversible chemical reaction when subjected to cyclic voltammetry.

27. A method according to claim 26 wherein the product of the irreversible chemical reaction displays reversible electrochemistry when the electrode is subjected to cyclic voltammetry.

28. A method according to claim 26 or claim 27 wherein the chemically sensitive redox active material undergoes polymerisation when subjected to cyclic voltammetry.

29. A method according to claim 27 or claim 28 wherein the chemically sensitive redox active material has a nitro group substituent.

AbstractELECTROCHEMICAL SENSORS

5 The invention provides an electrode for use in an electrochemical sensor (particularly a pH sensor), said electrode comprising carbon modified with a chemically sensitive redox active compound which is not anthraquinone, phenanthrenequinone or N,N'-diphenyl-*p*-phenylenediamine (DPPD). The invention further provides a pH sensor comprising:

10 a working electrode comprising carbon modified with a chemically sensitive redox active material; and
a counter electrode,

wherein the ratio of the surface area of the working electrode to the surface area of the counter electrode is from 1:10 to 10:1. Also provided is a pH sensor comprising:

15 a working electrode comprising carbon modified with a chemically sensitive redox active material, and
a counter electrode,

wherein the area of the working electrode is from $500\mu\text{m}^2$ to 0.1m^2 . The uses of these electrodes and sensors are also described.

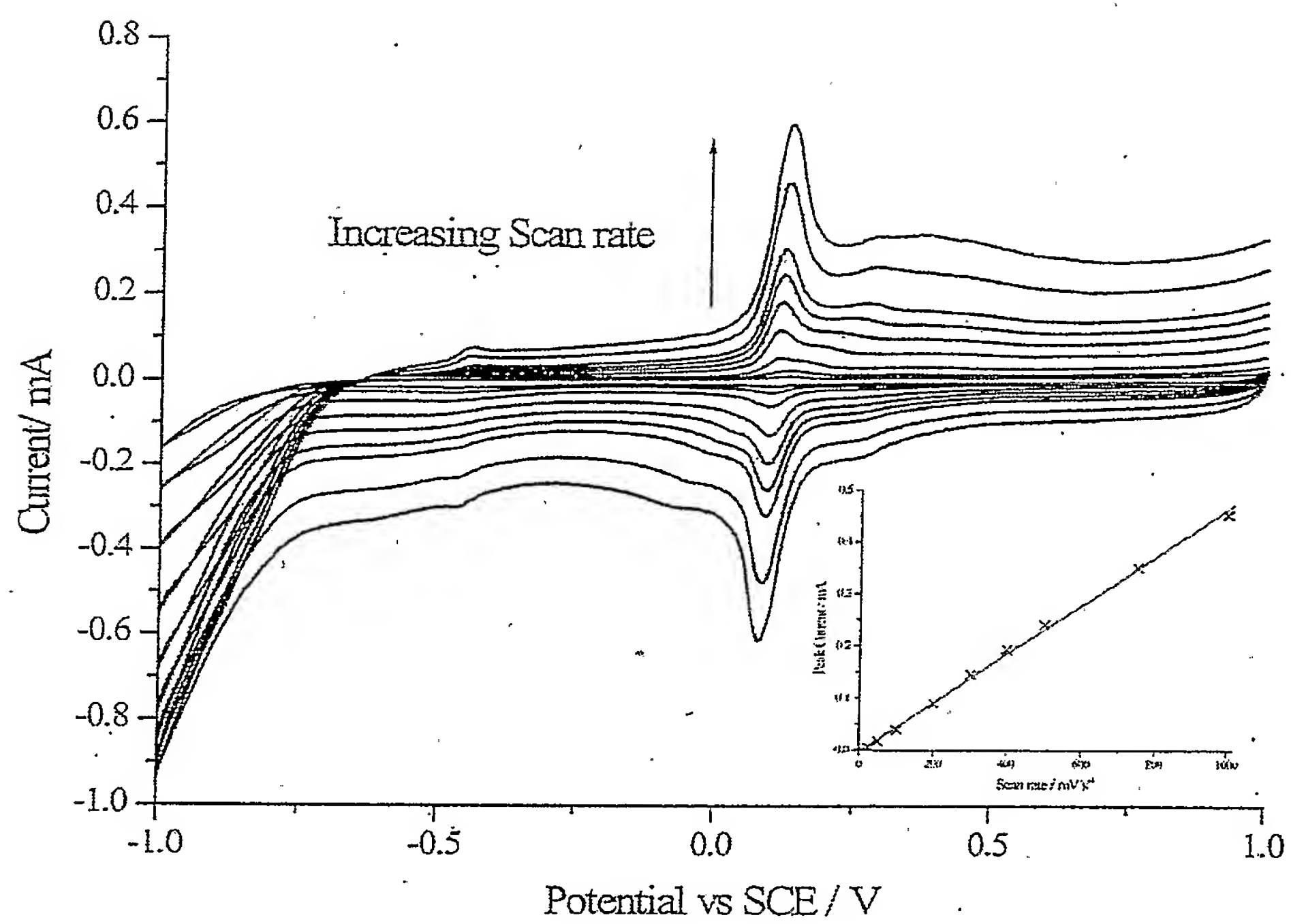


Figure 1

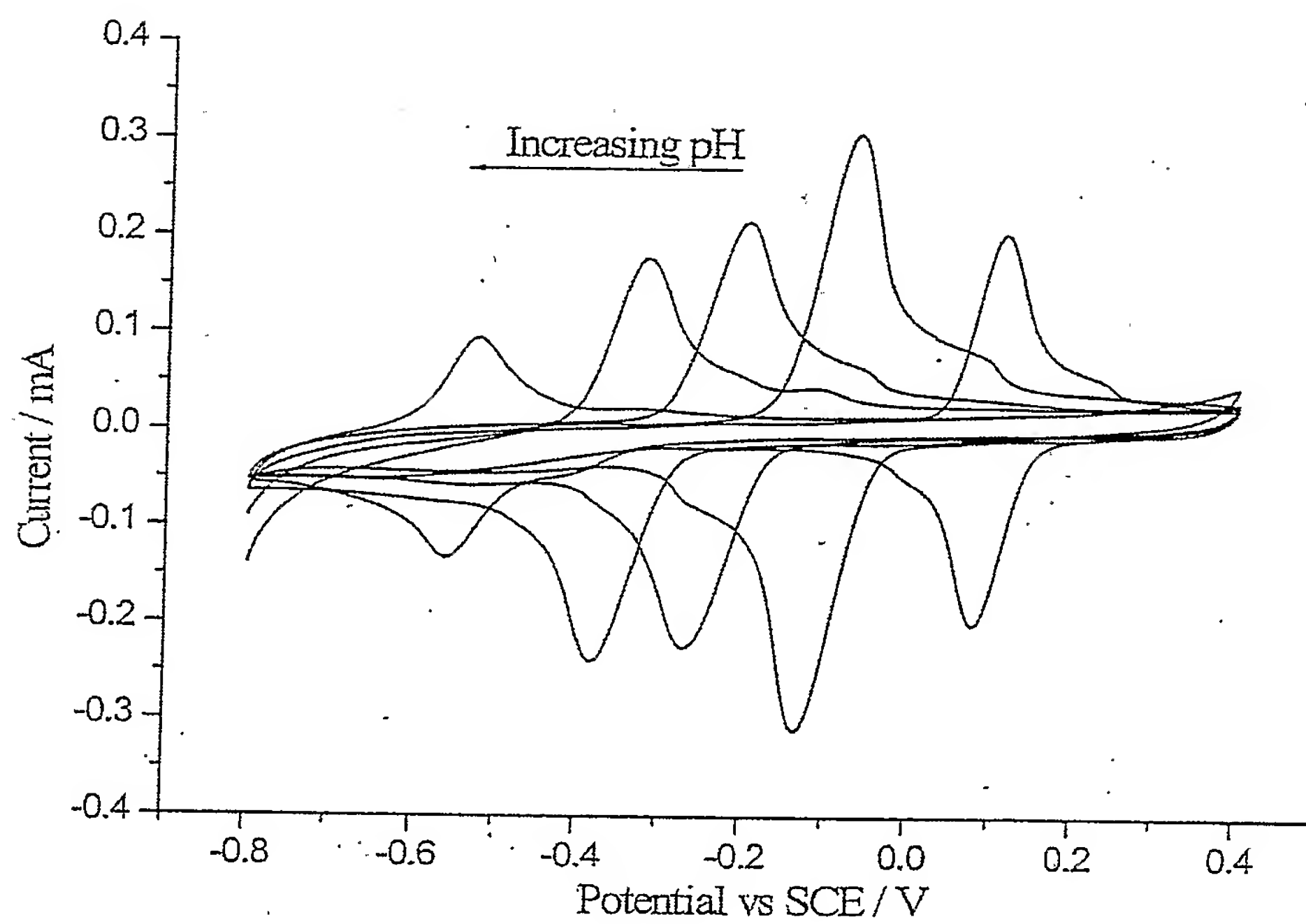
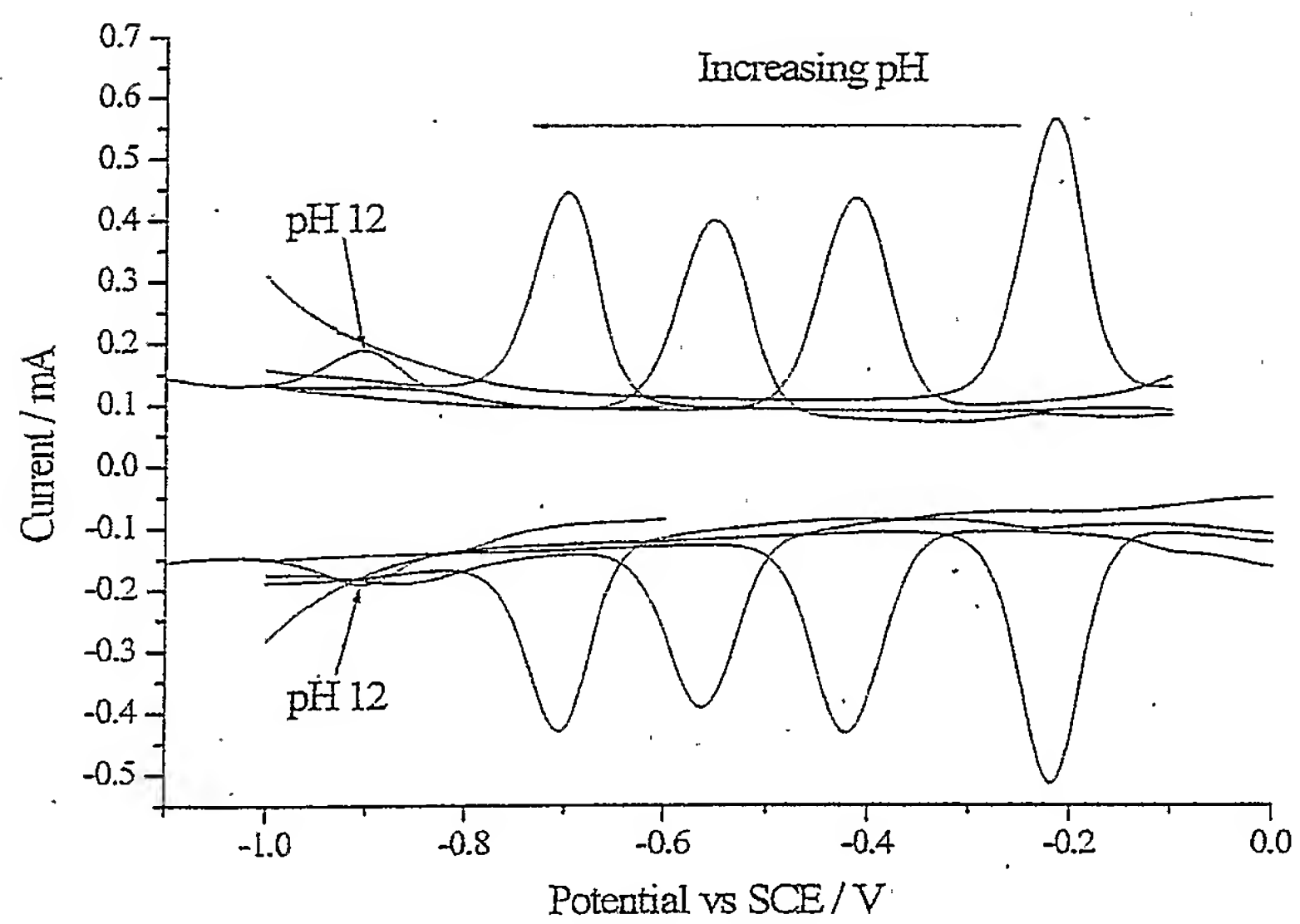
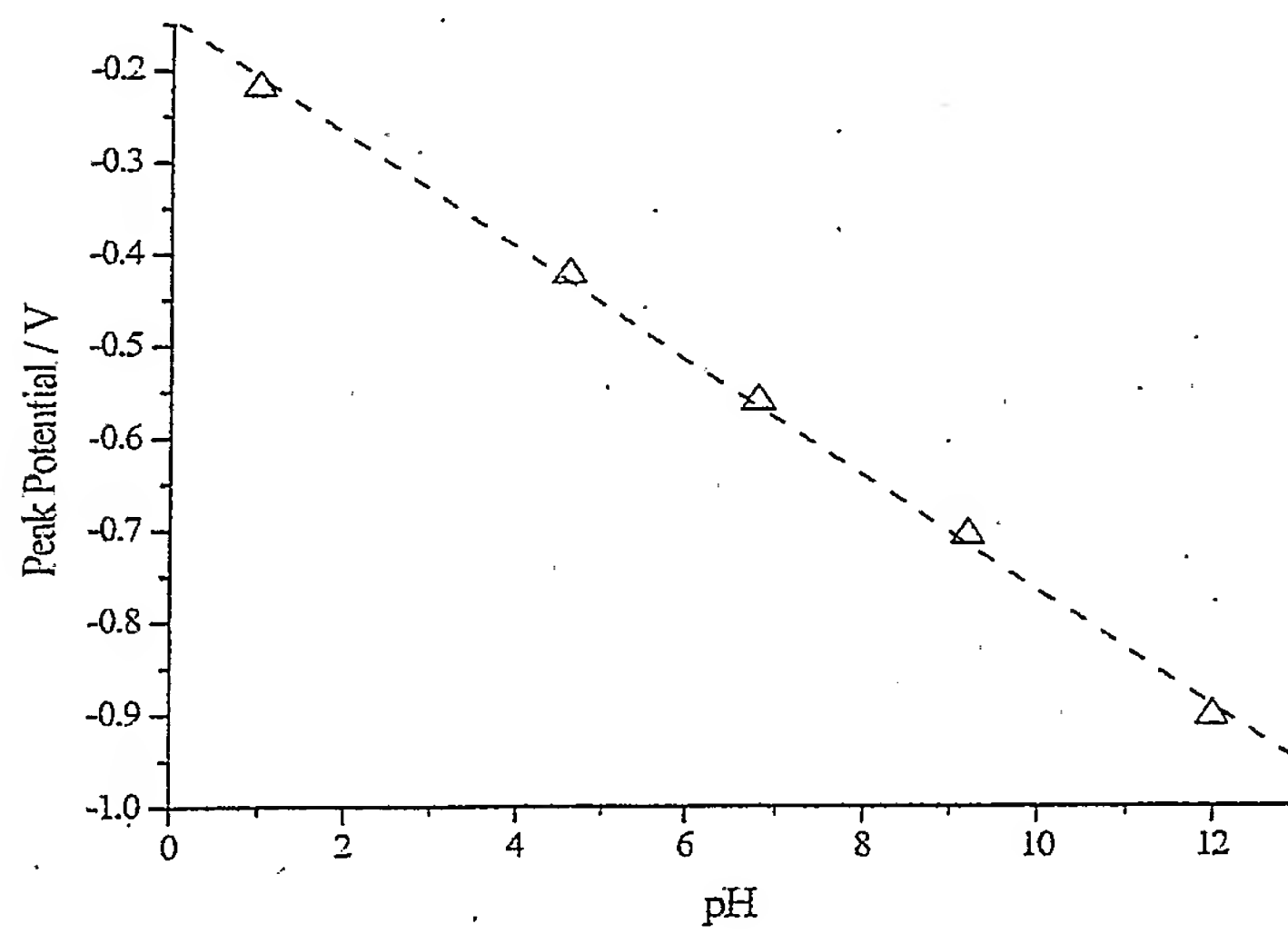


Figure 2

A)



B)



Figures 3a & 3b



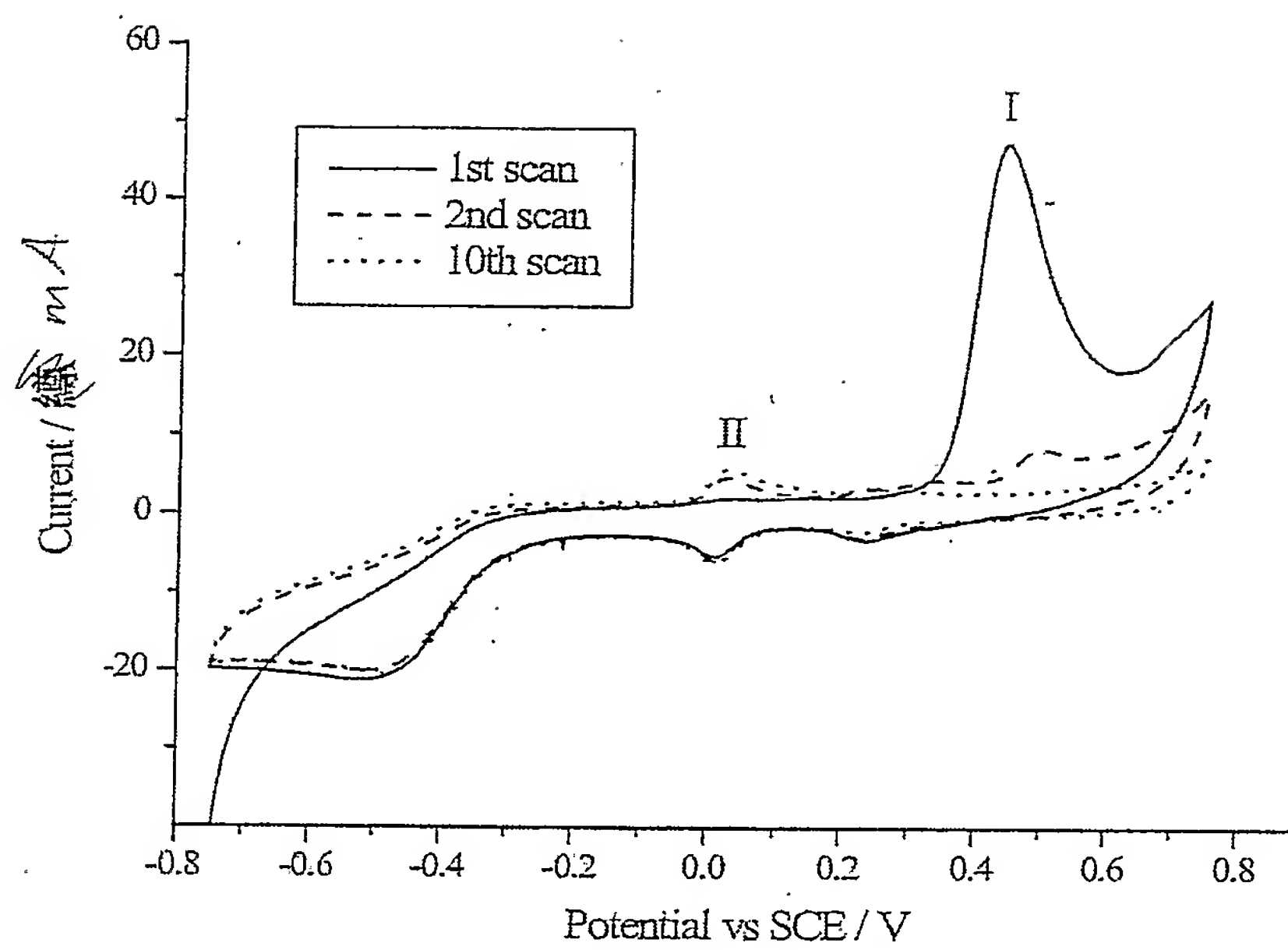


Figure 4



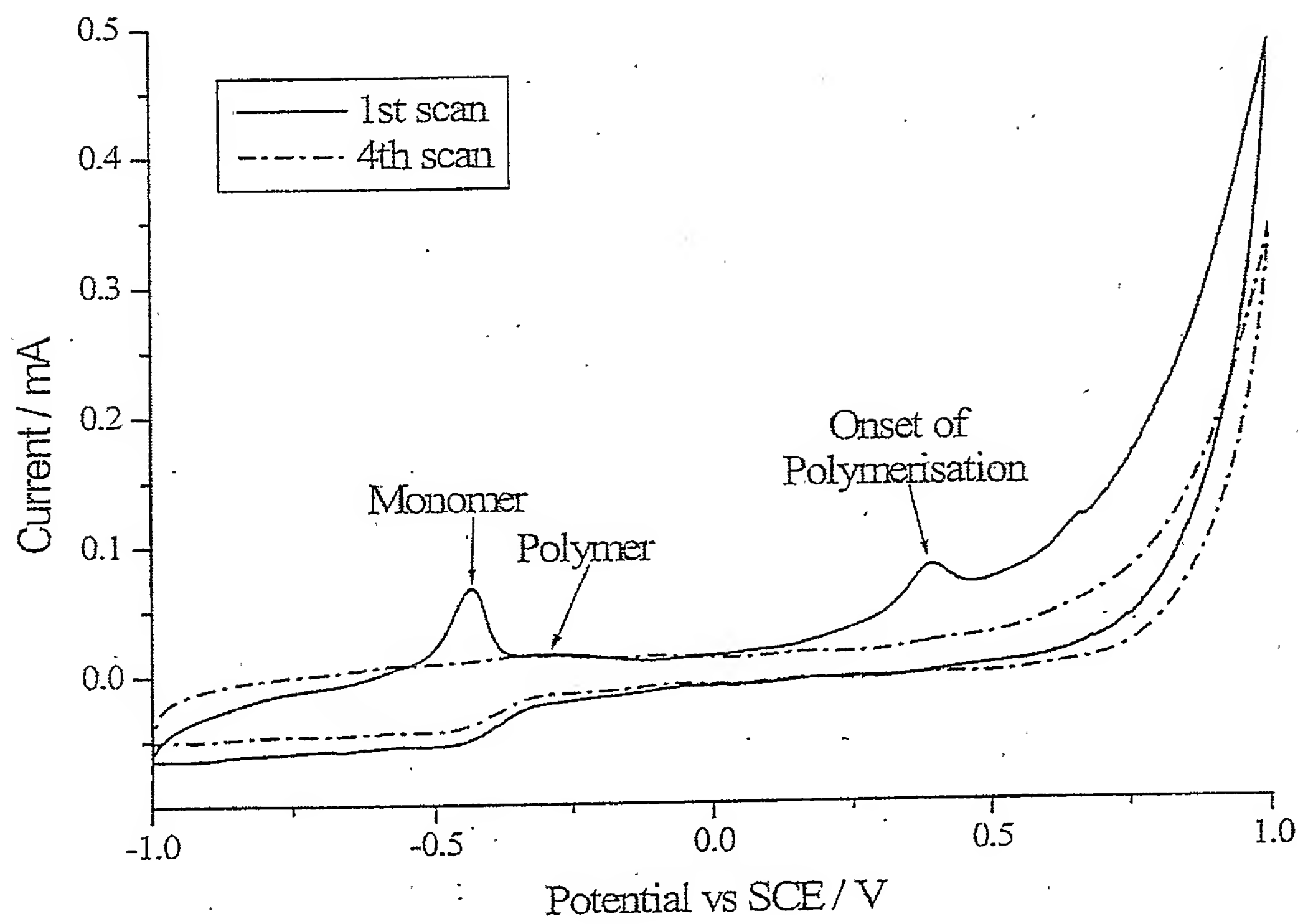


Figure 5



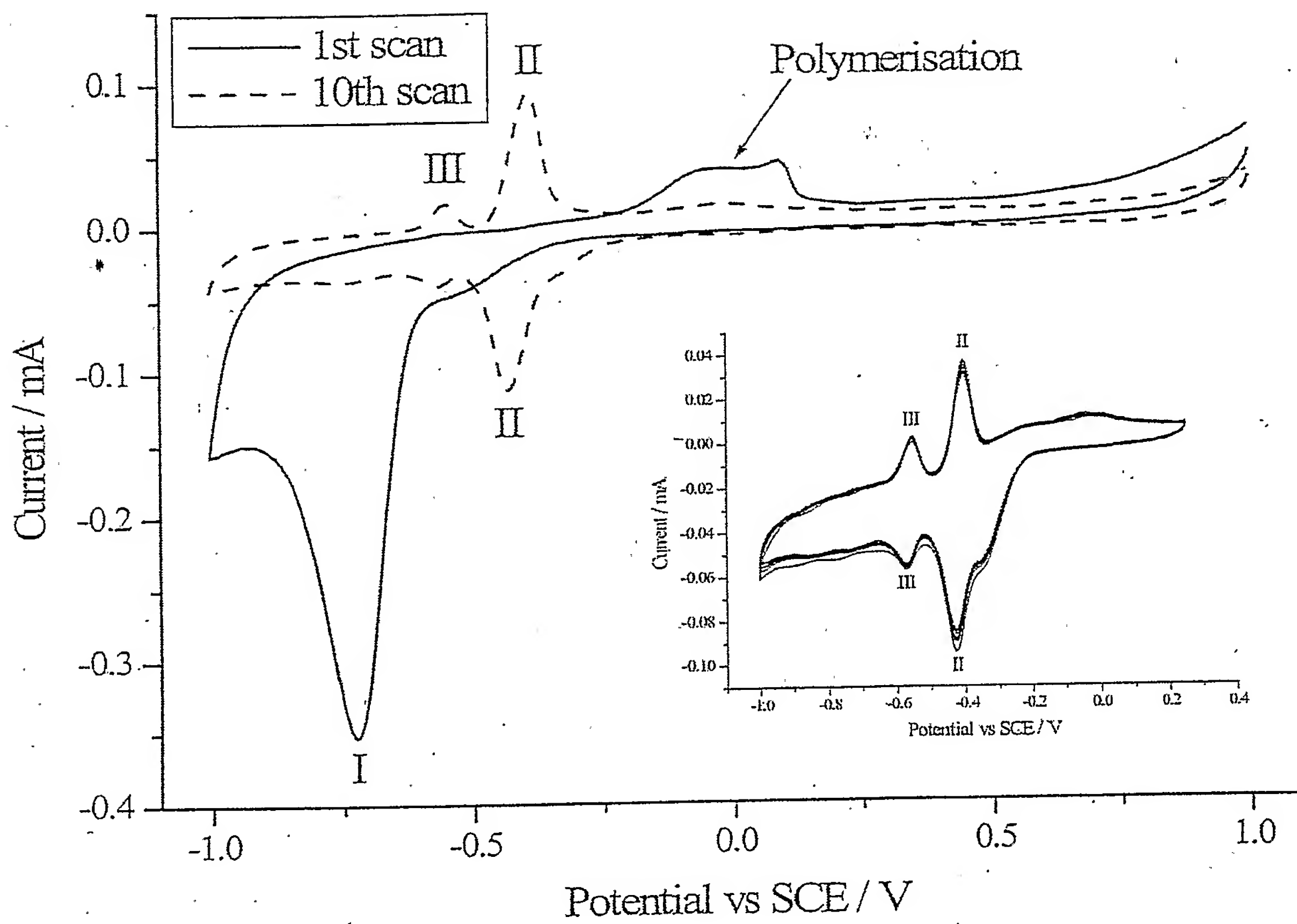


Figure 6



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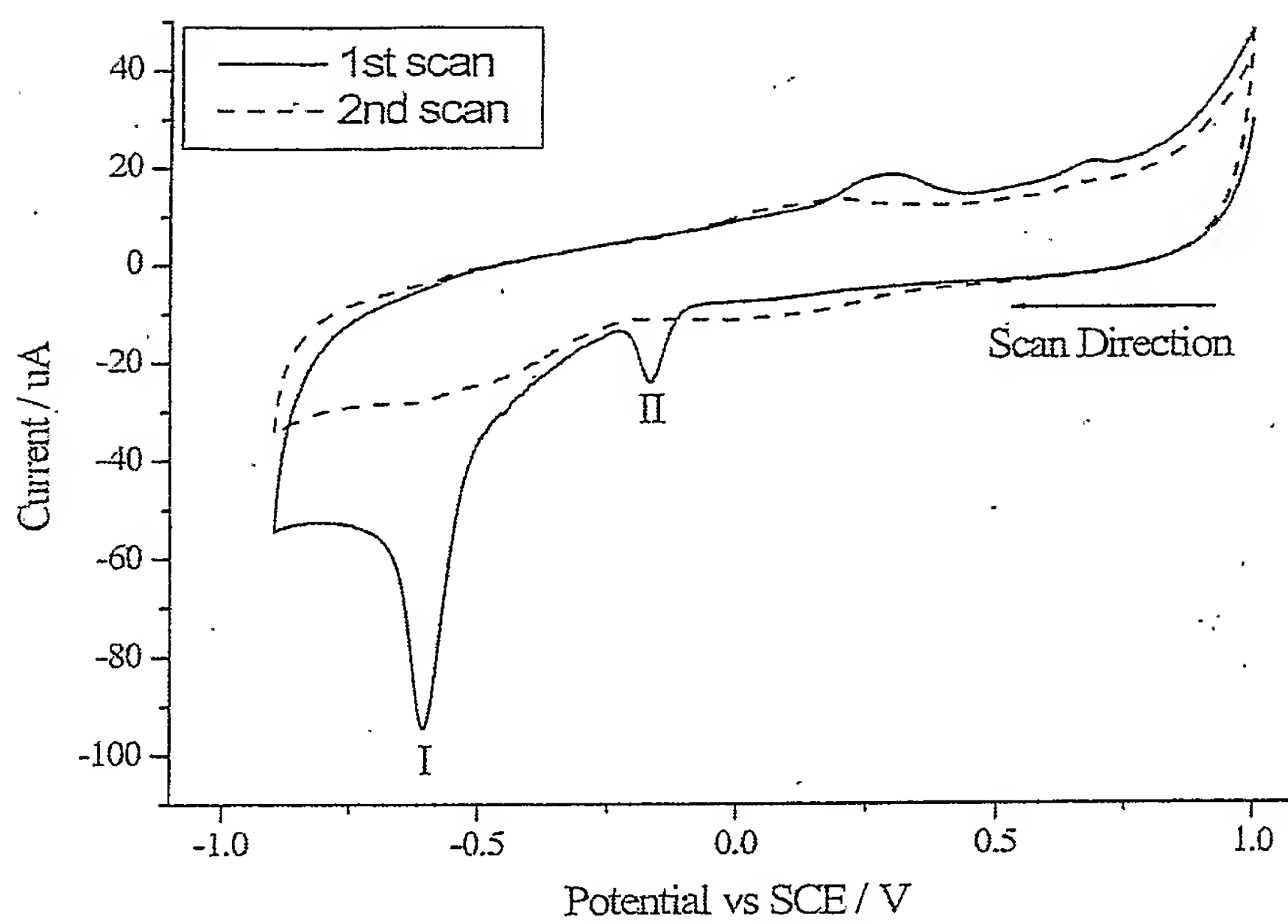


Figure 7



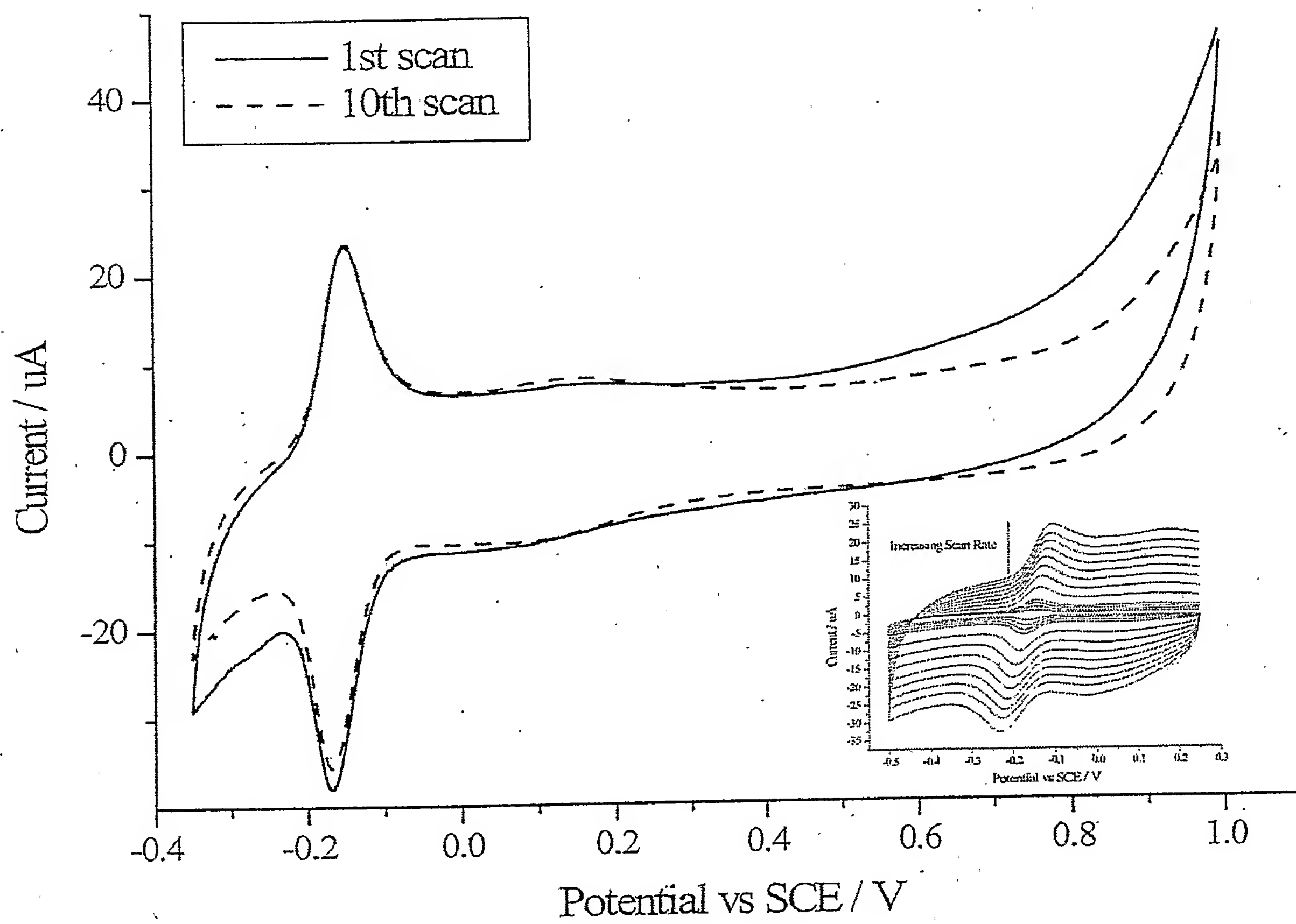


Figure 8



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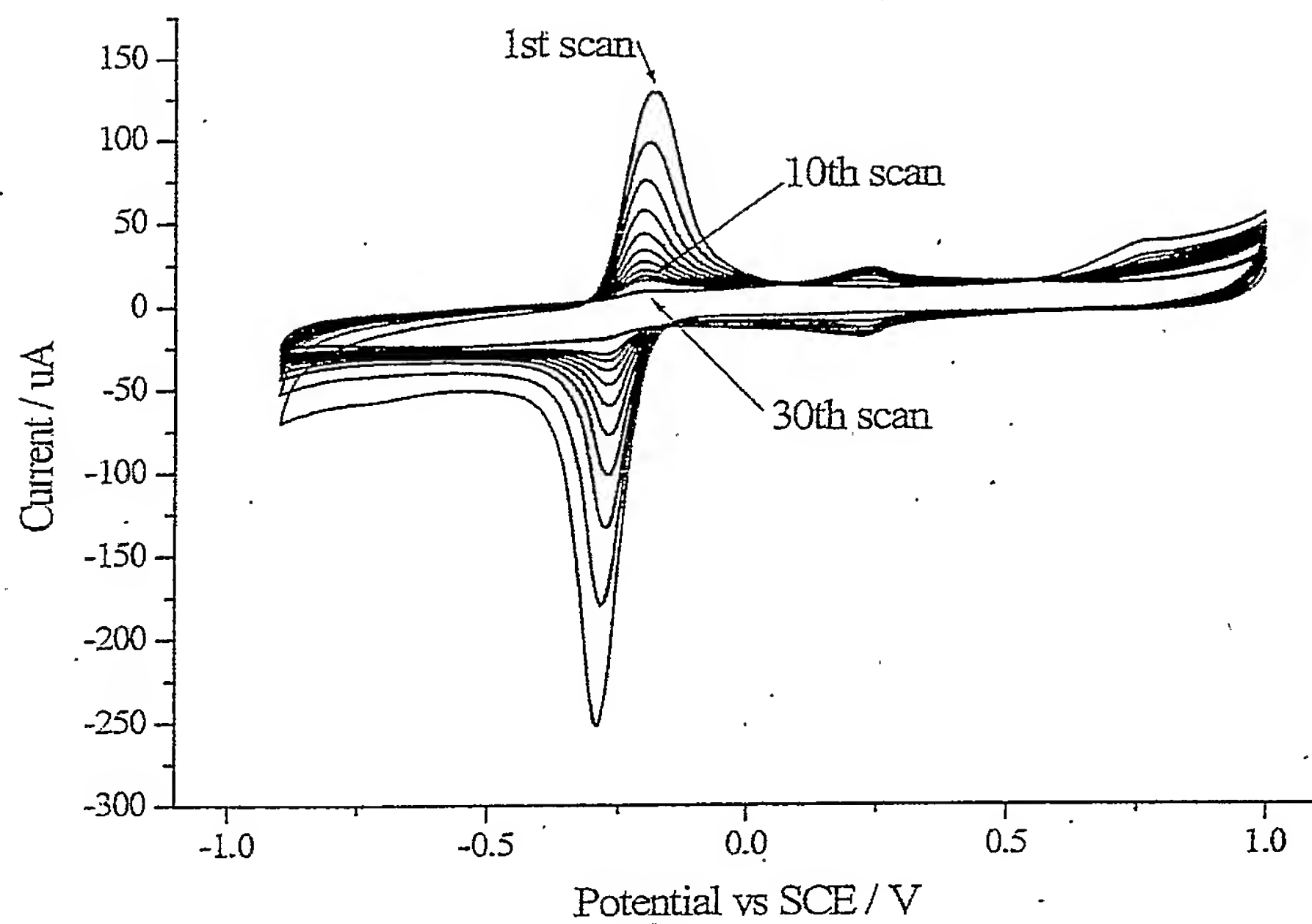


Figure 9



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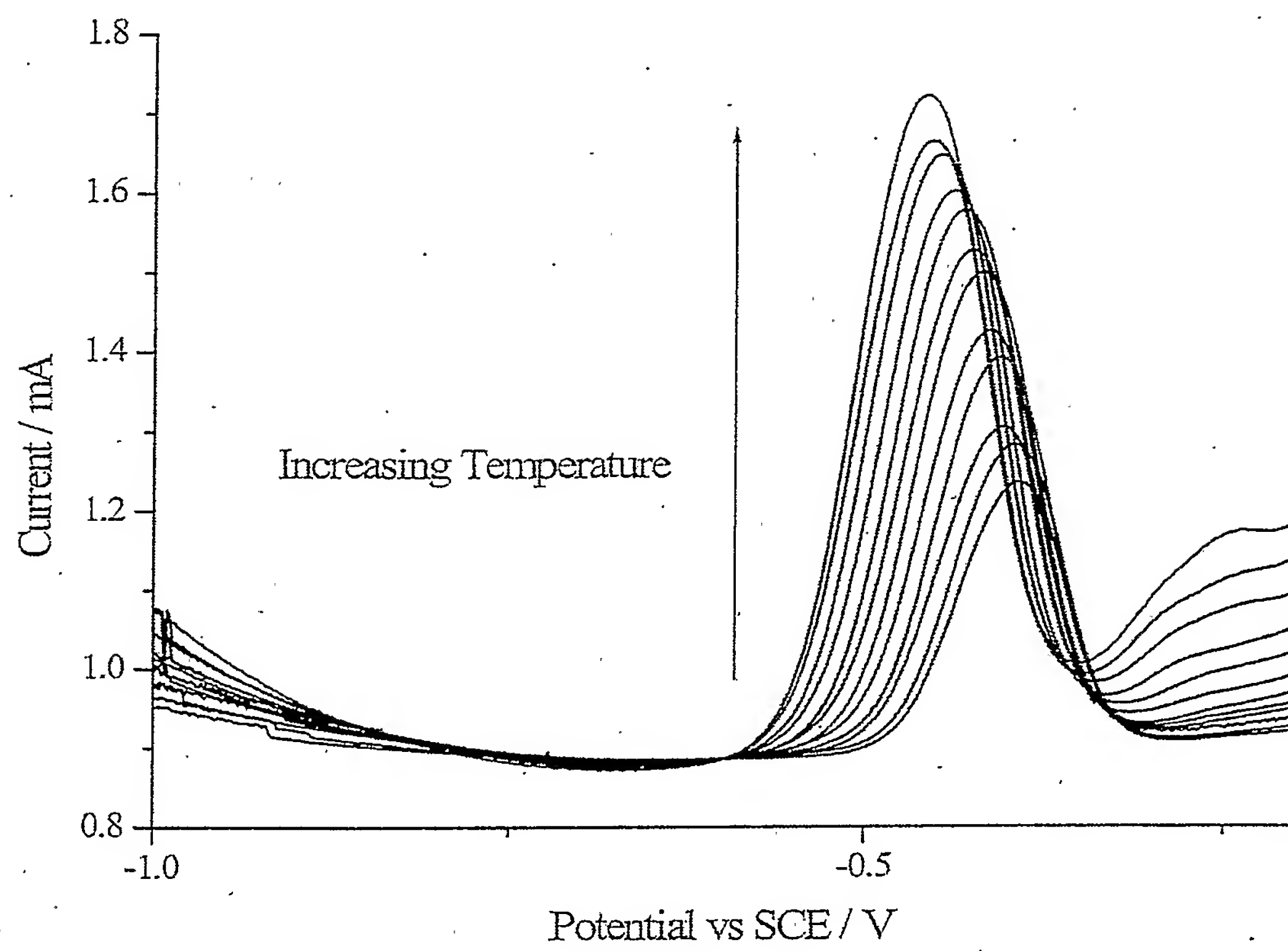


Figure 10



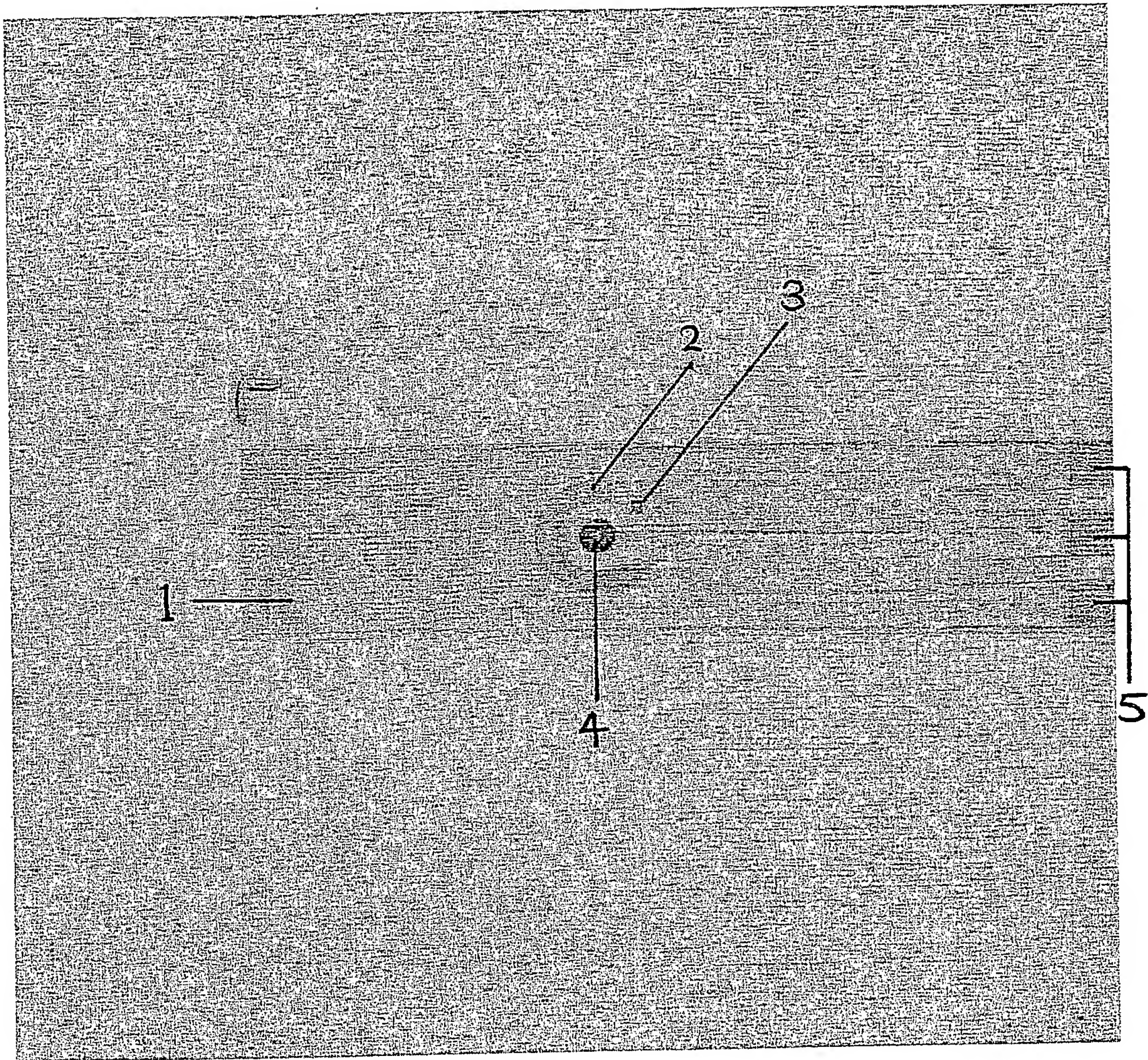


Figure 11

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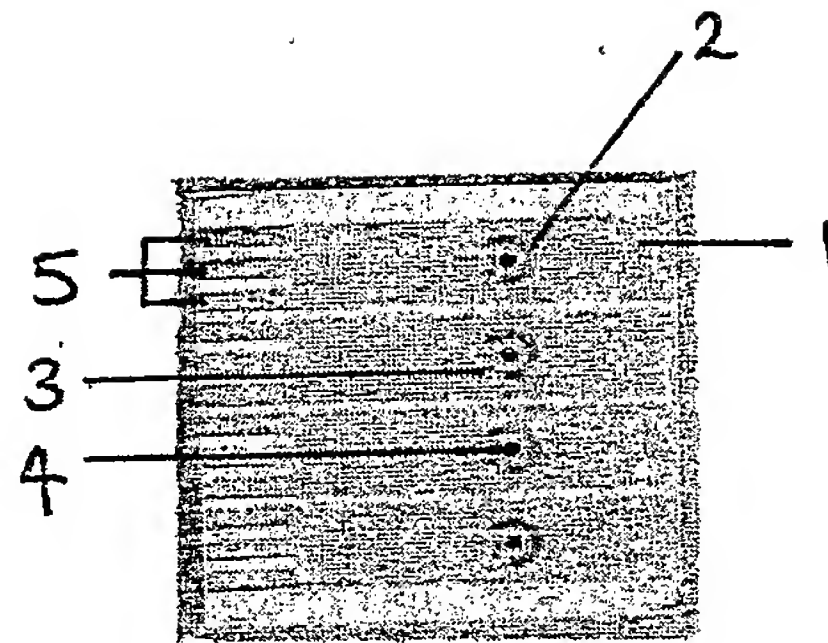


Figure 12

